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National Center for Genetic Engineering and Biotechnology (BIOTEC)

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BIOTEC has always strived to find a balance between excellence and relevance. In Thailand, science is not intrinsically perceived as relevant, so it is always gratifying when we can see our research results make their ways to actual users. This year, we launched a collaborative project with the Department of Agricultural Extension to transfer the effective *Beauveria bassiana* strain and the cultivation technology to the field stations of the Department, as well as the training of farmers to effectively produce the fungal spores from the inoculum distributed by the Department. The effective strain and cultivation process were the fruit of our research program to find useful applications for the wealth of the country’s biodiversity. *B. bassiana* has proven to be a potent biopesticide against insect pest larvae.

BIOTEC is currently undertaking two large-scale projects under NSTDA Giga Impact Initiative, a new program set up in 2013 providing funds to projects with high potential to generate a significant impact to the economy. One project aims to use genomic technologies in a sugarcane breeding program to increase yield and sugar level. The other project aims to develop a platform for large-scale production of industrial enzymes, concentrating on three recombinant enzymes for three distinct industries, namely animal feed, textile and pulp and paper. In addition to the R&D component, both projects are engaging industrial partners to expedite the transfer of technology to the private sector once completed. The sugarcane project is co-funded by and co-investigated with an industrial partner, Mitr Phol Group, a key player in sugarcane and sugar business in Thailand and the Asia Pacific; whereas the enzyme team is now in discussion with various enzyme users for field validation and commercial enzyme production in Thailand.

Human capital is the most important asset of any organization. This year, a number of our staff have brought pride to BIOTEC. Just a few examples illustrating the strength and diversity of our human resources; Dr. Bunpote Siridechadilok was named the 2014 Young Technologist by the Foundation for the Promotion of Science and Technology under the Patronage of His Majesty the King for the development of a quick and efficient method to construct mutations of dengue virus which will enable high throughput screening of drug targets as well as understanding gene functions. Dr. Noppol Kobmoo won the Marie Skłodowska-Curie Individual Fellowship to work with Prof. Titiana Giraud at the University of Paris-Sud, to use population genomics approach to explain the host specificity in insect fungi. In addition, Dr. Chalermpol Kirdmanee and Ms. Phongphan Ek-arwut were recognized for their work in the rural areas. Dr. Chalermpol received the Siam Cement Group Chair Professor Scholarship for the work on land rehabilitation in northeastern Thailand. The National Office of Buddhism honored Ms. Phongphan for her support in education of monastic schools in the rural area. Indeed, we never forget to be relevant.

This year, the Ministry of Science and Technology has been regrouped with the economic ministries, a good indication that our policy makers see the potential of science and technology. It also comes with responsibilities to deliver innovations to support our local industry. At BIOTEC, we are looking forward to making this contribution in the years to come.
BIOTEC was first set up under the Ministry for Science, Technology and Energy on 20 September 1983. After the establishment of the National Science and Technology Development Agency (NSTDA) on 30 December 1991, BIOTEC became one of the NSTDA centers, operating outside the normal framework of civil service and state enterprises. This enabled the Center to operate more effectively to support and transfer technology for the development of industry, agriculture, natural resources, environment and consequently the social and economic well-being of Thai people. Other centers under the NSTDA family include National Metal and Materials Technology Center (MTEC), National Electronics and Computer Technology Center (NECTEC), National Nanotechnology Center (NANOTEC) and Technology Management Center (TMC).

As a premier research institute in Thailand and Asia, BIOTEC operates research units located at Thailand Science Park and specialized laboratories hosted by various universities, covering a wide spectrum of research topics from agricultural science to biomedical science and environmental science. In addition to research units, development units have been established for activities with high commercial potential. These are full scale business and production operations designed to demonstrate the commercial viability of technologies to prospective investors.

Apart from research and commercialization, BIOTEC activities also include policy research, an outreach program, human resource development and international relations.
Collaborative Research Laboratories at universities and government organization

- Biochemical Engineering and Pilot Plant Research and Development Laboratory - at King Mongkut’s University of Technology Thonburi (KMUTT)
- Waste Utilization and Management Laboratory - at King Mongkut’s University of Technology Thonburi (KMUTT)
- Cassava and Starch Technology Research Laboratory - at Kasetsart University
- Rice Gene Discovery Laboratory - at Kasetsart University
- Medical Biotechnology Research Laboratory - at Faculty of Medicine Siriraj Hospital and Chiang Mai University
- Biomedical Technology Research Laboratory - at Chiang Mai University
- Marine Biotechnology Laboratory - at Chulalongkorn University
- Molecular Biology and Genomics of Shrimp Laboratory - at Chulalongkorn University
- Shrimp Molecular Biology and Biotechnology Laboratory - at Mahidol University
- Peat Swamp and Hala-Bala Rain Forest Research Laboratory - jointly established with the National Park, Wildlife and Plant Conservation Department and located in Narathiwat Province

Administration

- Policy Study and Biosafety Unit
- Rural Development Technology Service Unit
- Biotechnology Business Development Division
- Human Resources and Platform Technology Development Division
- Strategic Planning and Organization Development Division
- Evaluation and Monitoring Division
- Research Unit Coordination Division
- Management Information System Division
- International Cooperation and Public Relations Division
- Building Management and Equipment Service Division
- General Management Division
Socio-economic impact of 5,270 m THB generated by 49 completed project

- **Investment generation**: 127 m THB
- **Revenue generation**: 4,540 m THB
- **Cost reduction**: 509 m THB
- **Import replacement**: 91 m THB

Income from sources outside NSTDA, 107.74 m THB

- **Research Fund**: 62.73 m THB
- **Contract/Collaborative Research**: 24.84 m THB
- **Technical Services**: 16.10 m THB
- **Workshop/Conference**: 1.32 m THB
- **Licensing Activity**: 1.34 m THB
- **Others**: 1.41 m THB

**Major Outputs**

- **Publications**: 233 papers, including 16 papers in non-citation index journals
- **Honors and Awards**: 12 awards and honors

**Intellectual Properties**

- **granted patent overseas**: 1
- **granted patent in Thailand**: 31
- **granted petty patents in Thailand**: 26
- **patent applications in Thailand**: 27
- **petty patent applications in Thailand**: 23
- **trade secret applications in Thailand**: 2

Expenditure, 775.7 m THB

- **Technology Transfer, 3%**
- **Human Resource Development, 4%**
- **Infrastructure, 8%**
- **Internal Management, 15%**
- **Research Management, 3%**
- **Platform Technologies, 11%**
- **Cross-cutting Technologies, 0%**
- **Bioresources and Community, 22%**
- **Energy and Environment, 1%**
- **Health and Medicine, 9%**
- **Agriculture and Food, 24%**

Human Resources, 579

- **PhD., 31%**
- **M.Sc., 37%**
- **B.Sc., 27%**
- **Below B.S., 5%**
BIOTEC’s R&D and technical program covers a wide range of topics. Plant biotechnology focuses on three economically important plants: rice, cassava, and oil palm. Animal biotechnology focuses on shrimp and dairy cows, whereas food biotechnology aims to improve and upgrade the processing and quality of fermented food, including topics such as food safety and risk assessment, food chemistry, and starter culture technology. Medical biotechnology focuses on tropical and emerging diseases such as malaria, tuberculosis, dengue fever and influenza. On environmental issues, BIOTEC gives emphasis to the study of microbial diversity and the preservation, use and conservation of bioresources. Biogas and other renewable energies are the focus in the energy research theme.
Highlights from Rice Biotechnology

Using pseudo-backcrossing scheme to expedite rice gene-pyramiding

Rice production in irrigated areas of Thailand has been frequently and strongly affected by abiotic stresses resulting from unfavorable climatic changes, such as flooding and drought, as well as by biotic stresses caused by bacterial leaf blight (BB), leaf/neck blast (BL) and brown planthopper (BPH). Therefore, new successful breeding lines must possess multiple types of resistance to both biotic and abiotic stresses, as well as demonstrating specific grain qualities and high yield. However, pyramiding multiple genes into a desirable genetic background can take years to accomplish.

To shorten the time, researchers redesigned the gene-pyramiding platform by integrating marker assisted selection into pseudo-backcross breeding. With this platform, they were successful in pyramiding five functional genes (xa5, Xa21, Sub1A-C, SSIIa, TPS) and three QTLs (qBph3, qBL1, qBL11) into the ‘PinK3’ genome background within only seven breeding cycles in four years. ‘PinK3’ is an aromatic, high-yielding, non-photoperiod-sensitive, high-amylose rice variety, but it is susceptible to BPH, BB, BL and submergence stresses. The new, improved lines have a high-yield phenotype that confers submergence tolerance and resistance to BPH, BB and BL. This is the first report describing the application of pseudo-backcrossing to significantly shorten the time required for gene/QTL pyramiding in an annual crop (rice).

This work was a collaborative effort between Rice Gene Discovery Laboratory and Kasetsart University.

Genetic characterization of the world’s best rice, Myanmar Paw San Hmwe

Paw San Hmwe (PSM) rice from Myanmar is among the best rated rice varieties. It was named the World Best Rice 2011. It is cultivated in many areas of Myanmar. Strong aroma, good taste and its elongation during cooking are its key characteristics. However, the molecular marker-based analysis of prominent traits and genetic/phenotypic characteristics in the Paw San rice has not been well studied.

The genetic characterization of PSM accessions obtained from the Myanmar germplasm bank was investigated. Thirty-one PSM accessions were genotypically characterized and their physical grain and cooking quality traits were studied. Specific gene markers associated with aroma, apparent amylose content (AAC) and alkali spreading value were used to determine the alleles carried by different PSM accessions. The characterization and grouping data of PSM accessions posted benefits to Myanmar seed banks, and these results can facilitate the utilization of PSM rice as a genetic resource in rice breeding programs.

This study was jointly conducted by researchers from Rice Gene Discovery Laboratory, Kasetsart University, Myanmar Department of Agricultural Research and Myanmar Agriculture Service.

Insight into brown planthopper resistance mechanisms in Thai Jasmine rice

In Thailand, brown planthopper (BPH; *Nilaparvata lugens*) infestation is one of the major annual disease outbreaks in rice crops, causing huge cultural and economic burdens to Thai Jasmine rice producers. Khao Dawk Mali 105 (KDM 105; KD), a well-known Thai Jasmine rice, is highly susceptible to BPH. Planthoppers damage rice directly through feeding and also by transmitting two viruses, rice ragged stunt virus and rice grassy stunt virus. Up to 60% yield loss is common in susceptible rice cultivars attacked by BPH.

To better understand the biological mechanism of infestation, researchers investigated the metabolomic responses to BPH infestation in Thai rice varieties. $^1$H NMR spectroscopy, combined with chemometrics, was used to analyze the polar metabolome from leaf extracts of Thai Jasmine rice and its BPH resistant isogenic lines with and without BPH infestation at various time points. The study identified, for the first time, several potential metabolic pathways for acclimatization and defense mechanisms against BPH infestation. These findings provide a valuable, first insight into BPH resistance mechanisms in Thai Jasmine rice.

This study was conducted by researchers from BIOTEC, Kasetsart University, Queen’s University Belfast (UK) and Beaumont Health System (USA).


Large-scale SNP discovery in cassava via transcriptome sequencing

Cassava is one of the most important crop species, having multiple uses including starch and being the main source of dietary energy in several less developed countries. Marker-assisted selection has become an essential tool in plant breeding. Single nucleotide polymorphism (SNP) discovery via transcriptome sequencing is an attractive strategy for genome complexity reduction in organisms with large genomes.

To find an alternative to chemical bacteriocides, scientists explored the use of bacteriophages as biological control agents for *R. solanacearum*. Fourteen phages infecting *R. solanacearum* were isolated from soil samples collected in tomato fields in Chiang Mai, Thailand. The phages showed different host ranges when tested against 59 *R. solanacearum* strains isolated from Thailand and Japan. These phages were characterized as nine podoviruses and five myoviruses based on their morphology. The podoviruses isolated in this study showed strong lytic activity and wide host ranges. Therefore, these phages have potential use in the decontamination of pathogen-infected field soils. Phage J2 demonstrated an ability to prevent bacterial wilt of tomato in pot experiments.

This study was a collaborative effort between Genome Technology Research Unit, Kasetsart University and Hiroshima University (Japan).


Highlights from Plant and Animal Biotechnology

The use of bacteriophage to control bacterial wilt

Bacterial wilt caused by *Ralstonia solanacearum* is one of the most devastating diseases of many economically important crops in Thailand such as ginger, pepper, tomato, potato and *Curcuma alismatifolia* Gagnep. At present, protection from losses by bacterial wilt is achieved mainly by early detection and subsequent eradication by destroying the host, usually by using chemical bacteriocides.
Researchers sequenced the transcriptome of 16 cassava accessions using the Illumina HiSeq platform and identified 675,559 EST-derived SNP markers. A subset of those markers was subsequently genotyped by capture-based targeted enrichment sequencing in 100 F$_{1}$ progeny segregating for starch viscosity phenotypes. A total of 2,110 non-redundant SNP markers were used to construct a genetic map. This map encompasses 1,785 cM and consists of 19 linkage groups. A major quantitative trait locus (QTL) controlling starch viscosity was identified and shown to coincide with the QTL previously reported for this trait. This work represents the first effort to perform capture-based targeted enrichment sequencing in cassava and illustrates the attractiveness of this approach for genotyping SNPs in predetermined genomic regions.

This study was a collaborative effort by researchers from Mahidol University and Genome Technology Research Unit.


**Genome-wide SNP discovery in oil palm using genotyping-by-sequencing approach**

With rapid advancement in sequencing throughput, together with an overall decrease in sequencing cost, next generation sequencing technologies have been applied to SNP identification in various plant species. However, it remains costly to employ whole-genome sequencing to evaluate multiple individuals in a mapping population, especially for organisms with large genomes such as oil palm. Reduced representation methods are extremely useful, not only because of their cost-reducing aspects, but also because many research questions can be answered with a small set of markers and do not require every base of the genome to be sequenced. Genotyping-by-sequencing (GBS) is an efficient strategy that can simultaneously detect and score tens of thousands of molecular markers.

Researchers employed GBS approach to perform a large-scale SNP discovery and genotyping of a mapping population in oil palm. Over 21,000 SNP markers were identified and 1085 markers were placed on the genetic map. This SNP-based linkage map was subsequently employed in a QTL analysis to detect markers associated with fruit bunch weight and trunk height. These markers will be useful for selecting individual palms with desirable characteristics in breeding programs. Furthermore, the high-density map will contribute to a fundamental knowledge of genome structure and will be valuable for mapping other economically important genes for marker-assisted selections.

This study was performed by Genome Technology Research Unit.


**Finding genes responsible for oil palm yield**

Oil palm (Elaeis guineensis), the tropical perennial and cross-pollinated crop, is the most productive oil-producing crop. Improvement of oil palm yield could significantly contribute to the overall oil production in the world. The number of fruit bunches is one yield component that can be improved by increasing sex ratio, the ratio of female inflorescences to total inflorescences.

Researchers attempted to identify gene(s) by making a physical map of a specific locus responsible for sex ratio. A putative aldo-keto reductase gene (named EgAKR1) was revealed to be a promising candidate for sex ratio determination, via controlling female inflorescence number. This was predicted from the two newly identified polymorphic marker loci (mEgSSRsr8-21LB and mEgAKR1-9) designed from EgAKR1. The functions of AKR gene families in other plant species and the promoter analysis suggested that EgAKR1 may contribute to the sex ratio through abiotic stress responsiveness.

This investigation was made by researchers from Genome Technology Research Unit and Thammasat University.

Research and Development

High-density integrated genetic linkage map of rubber tree

Construction of linkage maps is crucial for genetic studies and marker-assisted breeding programs. Recent advances in next generation sequencing technologies allow for the generation of high-density linkage maps, especially in non-model species lacking extensive genomic resources.

Researchers employed the genotyping-by-sequencing (GBS) technique to perform a genome-wide SNP discovery and genotyping of two rubber tree mapping populations. Single-population linkage maps were generated and common SNP markers were used as bridges to merge them into a high-density integrated genetic map, possibly the most saturated genetic map on rubber tree to date. SNP markers reported in this study will expand the existing repertoire of available molecular markers in rubber tree, and the integrated genetic map presented will be useful for future breeding programs, association studies with desirable agronomic traits, genetic diversity analyses and phylogenetic studies. This study also demonstrated that GBS is a robust and cost-effective approach for generating a common set of genome-wide SNP data suitable for constructing integrated linkage maps from multiple populations in a highly heterozygous agricultural species.

This was a collaborative work between Genome Technology Research Unit and Rubber Research Institute of Thailand.


Development of porcine epidemic diarrhoea vaccine

Porcine epidemic diarrhoea virus (PEDV) causes acute diarrhoea and dehydration in swine of all ages, with significant mortality in neonatal pigs. The recent rise of PEDV outbreaks in Asia and North America warrants an urgent search for effective vaccines. However, PEDV vaccine research has been hampered by difficulties in isolating and propagating the virus in mammalian cells, thereby complicating the recovery of infectious PEDV using a full-length infectious clone.

In this study, researchers engineered VeroE6 cells to stably express porcine aminopeptidase N (pAPN) and used them as a platform to obtain a high-growth variant of PEDV, termed PEDV_{AVCT12}. Subsequently, the full-length cDNA clone was constructed by assembling contiguous cDNA fragments encompassing the complete genome of PEDV_{AVCT12}.

GBS-based genetic linkage maps of rubber tree derived from F_1 progeny from (A) BPM24 × RRIM600 and (B) BPM24 × RRIC110 crosses.

Porcine epidemic diarrhoea virus has a substantial economic burden given that it is highly infectious, resulting in significant morbidity and mortality in piglets.
in a bacterial artificial chromosome. Infectious PEDV could be recovered, and the rescued virus displayed phenotypic properties identical to the parental virus. Interestingly, PEDVAVCT12 was found to contain a C-terminal deletion of the spike gene, resulting in disruption of the ORF-3 start codon. When a functional ORF-3 gene was restored, the recombinant virus could not be rescued, suggesting that ORF-3 could suppress PEDV replication in vitro. In addition, a high-growth and genetically stable recombinant PEDV expressing a foreign protein could be rescued by replacing the ORF-3 gene with the mCherry gene. Together, the results of this study provide a means to generate genetically defined PEDV as a promising vaccine candidate.

This study was conducted by Animal Biotechnology Research Unit.


### Highlights from Shrimp Biotechnology

#### Study of shrimp growth and reproduction

Closing life cycle culture is crucial to the domestication and genetic improvement of the giant tiger shrimp (*Penaeus monodon*). However, poor reproductive maturation of captive *P. monodon* females and low quality of spermatozoa of captive males have limited the potential of genetic improvement, which in turn, resulted in remarkably slow domestication and selective breeding programs of *P. monodon* in Thailand.

Researchers investigated *PmFAME* gene which is believed to regulate growth and reproduction of *P. monodon*. Two full-length cDNAs of PmFAME were identified, in order to examine molecular involvement of PmFAME gene products on ovarian (and oocyte) development. PmFAME was found to be differentially expressed during ovarian development of *P. monodon*. Eyestalk ablation and exogenous serotonin injection can promote PmFAME expression.

In a separate study, researchers evaluated biological roles of *X-box binding protein 1* in reproduction and growth of *P. monodon* by characterizing the *PmXbp1* cDNA sequence. Expression patterns of *PmXbp1* during ovarian development in wild *P. monodon* broodstock were examined. The study showed that eyestalk ablation had an effect on the expression level of PmXbp1 during late ovarian development, whereas serotonin injection promoted the expression level of ovarian PmXbp1.

Both studies were conducted by Animal Biotechnology Research Unit in collaboration with Chulalongkorn University.

Ref:

#### Transmission of yellow head virus

Yellow head virus (YHV) is an economically important disease in farmed shrimp in South East Asia. Despite the continual search since its discovery in the early 1990s, the reservoir for YHV-1, the most virulent strain of YHV found in Thailand, has still not been identified. Outbreaks of the virus in cultivated, exotic white leg shrimp *P. vannamei* that originate from SPF stocks known to be free of the virus, suggested that the outbreaks occur via horizontal transmission from an environmental source. Since the Australian red claw crayfish, *Cherax quadricarinatus* and the marine penaeid shrimp are often cultivated in adjacent areas in Thailand, it is of importance to know whether red claw crayfish is susceptible to YHV and whether YHV could be transmitted between these species.

Examples of red claw crayfish and shrimp gill tissues immuno-stained with monoclonal antibodies against YHV nucleocapsid protein and counter stained with hematoxylin and eosin.
Researchers performed the tests whether red claw crayfish was susceptible to endemic YHV and also capable of transmitting it to black tiger shrimp. The results revealed that red claw crayfish is susceptible but highly tolerant to the virus. Infected red claw crayfish could transmit the virus without showing any signs of yellow head disease. These facts make them a perfect carrier for the virus.

This study was conducted by researchers from BIOTEC Shrimp Molecular Biology and Biotechnology Laboratory, Mahidol University, Institute of Veterinary Research and Development of Central Vietnam and Institute of Biotechnology (Vietnam).

Ref: Soowannayan, C., Nguyen, G.T., Pham, L.N., Phanthura, M. and Nakhong, N. (2015). Australian red claw crayfish (Cherax quadricarinatus) is susceptible to yellow head virus (YHV) infection and can transmit it to the black tiger shrimp (Penaeus monodon). Aquaculture, 445, 63-69.

Immunostimulant effect of copra meal MOS on white shrimp

Mannooligosaccharides (MOS) are linear chains of mannose sugars. They are known to possess prebiotic affect and therefore can be used as a feed additive to prevent pathogen colonization and modulate the immune system of host animals. Most MOS used in animal feed additives are derived from the cell wall of yeast. However, copra meal, a waste product from coconut industries, is another rich source for MOS.

BIOTEC researchers investigated the effects of MOS from copra meal as a dietary feed additive on growth performance, disease resistance, and immune enhancement of the Pacific white shrimp Litopenaeus vannamei. The findings showed that MOS from copra meal was able to significantly increase protection in the Pacific white shrimp upon pathogen exposure, while it had no significant effect on shrimp growth rate and feed conversion ratio. Moreover, MOS supplementation induced crucial shrimp immune-related genes associated with antimicrobial peptides and the peritrophic membrane. The findings suggested that MOS can potentially be applied as an immunostimulant, particularly in aquaculture, and copra meal, which is considered a waste product of the coconut industry, can be an alternative source for MOS.

This study was conducted by researchers from Food Biotechnology Research Unit and Biosensing Technology Research Unit.


Highlights from Food Science and Biotechnology

Discovery of a halotolerant yeast, a potential starter culture for soy sauce fermentation

Soy sauce is made by the fermentation of soybeans combined with wheat flour, rice flour and brine. The production involves two steps: koji fermentation using Aspergillus oryzae and moromi fermentation by adding brine solution into the koji. In moromi fermentation, glutaminase produced by naturally-present yeast converts L-glutamine originated from soy protein to L-glutamic acid, a compound responsible for “umami” taste. Given the high salinity condition of moromi fermentation, a halotolerant yeast would be beneficial to the soy sauce fermentation.

Researchers investigated yeast isolates obtained from Thai soy sauce fermentation. The most interesting yeast was identified as Meyerozyma (Pichia) guilliermondii EM2Y61. This strain is a salt-tolerant yeast that could tolerate up to 20% (w/v) NaCl and produce extracellular and cell-bound glutaminases. The extracellular glutaminase activity was found to be much higher than that of cell-bound glutaminase. This is the first report of glutaminase producing M. guilliermondii isolated from the moromi of Thai soy sauce fermentation. M. guilliermondii EM2Y61 has high potential to be developed into starter yeast culture to increase L-glutamic acid during soy sauce fermentation.

This was a joint study between Mahidol University and Food Biotechnology Research Unit.

Cassava pulp, waste from starch factory, can be a potentially good source of prebiotics, because of its high hemicelluloses content that promotes the growth of gut flora.
Researchers isolated a phage from an infected culture of *B. amyloliquefaciens* FB11, a strain used locally in Thailand. The phage was classified morphologically into the Caudovirales order, Myoviridae family. In order to eliminate phage infection, an early detection of the phage and an inactivation measure must be applied in combination. Researchers therefore developed a PCR-based method that can detect the phage with a $10^4$ PFU/mL limit of detection, in less than 3 h including sample treatment, PCR analysis and gel electrophoresis. The study also demonstrated that the phage could be inactivated by either thermal treatment at 70°C for 5 min or treatment with peracetic acid-based disinfectant (0.3 % v/v) for 5 min.

This work was conducted by Food Biotechnology Research Unit.

**Prebiotics from rice bran and cassava pulp**

Agricultural wastes such as rice bran and cassava pulp are inherently rich in plant cell wall materials like hemicelluloses, which are complex heteropolymers consisting of several kinds of monosaccharides connected by various glycosidic linkages. Due to this variety of glycosidic bonds between the monomers, hemicelluloses are usually not digestible by human intestinal digestive enzymes, but could be utilized by members of the intestinal microbiota. The fact that hemicelluloses can be utilized by some beneficial microbes present in the intestine makes hemicellulose-containing substances potential prebiotic sources.

Researchers investigated the prebiotic potential of rice bran and cassava pulp. Hydrothermal treatments were used to extract hemicellulosic oligosaccharide mixtures from rice bran and cassava pulp. Microbial utilization of the obtained oligosaccharide mixtures showed that they were able to promote the growth of two from three *Lactobacillus*, as well as three from five *Bifidobacterium* species tested. From the three tested *Bacteriodes* strains, one utilized the cassava pulp oligosaccharide mixture better than inulin, while two grew better on rice bran oligosaccharide mixture than on inulin. Furthermore, the two oligosaccharide mixtures were found to be stable at 85°C for 30 min. Similarly, these mixtures were able to withstand their exposure to simulated human gastric juice (pH 1–5).
and to pancreatin treatments for up to two hours. These findings suggested that rice bran and cassava pulp can be a potentially good source of prebiotics.

This research was a joint study between Mahidol University and Food Biotechnology Research Unit.


Molecular characterization of Plasmodium falciparum Bruno/CELF RNA binding proteins.

The human malaria parasite Plasmodium falciparum employs intricate post-transcriptional regulatory mechanisms in different stages of its life cycle. Despite the importance of post-transcriptional regulation, key elements of these processes, namely RNA binding proteins (RBPs), are poorly characterized.

Researchers characterized the RNA binding properties of P. falciparum proteins, including two putative members of the Bruno/CELF family of RBPs (PfCELF1 and PfCELF2), dihydrofolate reductase-thymidylate synthase (PfDHFR-TS), and adenosine deaminase (PfAda). RNA binding activity was tested using UV-crosslinking and electrophoretic mobility shift assays. PfCELF1 and PfDHFR-TS demonstrated RNA binding activity, whereas PfAda and PfCELF2 were RBP-negative. Intracellular protein localization of RBPs was studied using GFP-tagged transgenic parasite lines. PfCELF1 protein may shuttle between nucleus and cytoplasm, as shown by a predominantly nuclear PfCELF1 cell population and another predominantly cytoplasmic. In contrast, PfDHFR-TS protein is predominantly cytoplasmic. PfCELF1 may thus have several roles, including pre-mRNA processing. The mRNA targets of these P. falciparum proteins were investigated by ribonomics using DNA microarrays. A sequence motif similar to that recognized by CELF proteins in other species is common in the introns of target mRNAs identified for PfCELF1, suggesting that nuclear-localized PfCELF1 may regulate pre-mRNA splicing in P. falciparum, as has been found for CELF proteins in other species. In contrast, none or very few mRNA targets were found for the other proteins, suggesting that they do not have biologically relevant roles as RBPs in the asexual stages of P. falciparum.

This work was conducted by Medical Molecular Biology Research Unit in collaboration with the University of Edinburgh.


Highlights from Malaria Research

Bacterial surrogate system: A tool to explore antimalarial drug interaction

With continuing global threat of malaria, there is an urgent need to search not only for new drugs, but also for effective drug combinations. Inhibitors of dihydrofolate reductase (DHFR) such as pyrimethamine and of dihydropteroate synthase (DHPS) such as sulfa drugs are known to have synergistic interactions. However, studies of the synergism are complicated by the fact that the malaria parasite can also salvage exogenous folates, and the salvage may also be affected by the drugs. It is desirable to have a convenient system to study interaction of DHFR and DHPS inhibitors without such complications.

Researchers used Escherichia coli transformed with malarial DHFR and DHPS, while its own corresponding genes were inactivated by optimal concentration of trimethoprim and genetic knockout, respectively, to study the interaction of the inhibitors. Marked synergistic effects are observed for all combinations of pyrimethamine and sulfa inhibitors in the presence of trimethoprim. The results show synergism between inhibitors of the two enzymes even in the absence of folate transport and uptake. This bacterial surrogate system can, therefore, be used as a tool for assessing the interactions of drug combinations between the DHFR and DHPS inhibitors.

This study was conducted by researchers from Mahidol University and Medical Molecular Biology Research Unit.

Crystal structure of *Plasmodium vivax* serine hydroxymethyltransferase

*Plasmodium* parasites, the causative agent of malaria, rely heavily on de novo folate biosynthesis, and the enzymes in this pathway have therefore been explored extensively for antimalarial development. Serine hydroxymethyltransferase (SHMT) from *Plasmodium* spp., an enzyme involved in folate recycling and dTMP synthesis, has been shown to catalyze the conversion of L- and D-serine to glycine (Gly) in a THF-dependent reaction, the mechanism of which is not yet fully understood.

In this study, researchers determined the crystal structures of *Plasmodium vivax* SHMT (PvSHMT) in a binary complex with L-serine and in a ternary complex with D-serine (D-Ser) and (6R)-5-formyltetrahydrofolate (5FTHF), which suggest the mechanism underlying the control of enzyme activity. The results assert the importance of features such as stereoselectivity and redox status for control of the activity and specificity of PvSHMT. The structure–function analysis of PvSHMT has led to the design of antimalarials targeting SHMT.

This work was a collaborative effort between researchers from Mahidol University, Burapha University, National Synchrotron Radiation Research Center (Taiwan), Medical Molecular Biology Research Unit and Biosensing Technology Research Unit.


Kinetic mechanism of *Plasmodium vivax* serine hydroxymethyltransferase

*Plasmodium* SHMT has been shown to be essential for parasite growth and development, making it a prime target for antimalarial drug chemotherapy development. An in-depth understanding of *Plasmodium* SHMT kinetics and mechanism is needed so that the differences in reaction details among SHMTs can be understood and used for the development of specific inhibitors.

Researchers employed various methodologies including ligand binding measurements, as well as transient and steady-state kinetics to investigate the PvSHMT reaction. The results suggested that the kinetic mechanism of PvSHMT occurs via a random-order model and that glycine formation is the rate-limiting step of SHMT. This information will serve as a basis for future investigation for other SHMTs. The comparative studies of SHMTs from parasite and host will pave the way for the design of species-specific inhibitors for antimalarial drug development.

This work was a collaborative effort between researchers from Mahidol University, Burapha University, Chulalongkorn University, the University of Michigan (USA), Medical Molecular Biology Research Unit and Biosensing Technology Research Unit.


**Highlights from Dengue Research**

Role of host protein in dengue virus replication

Host and viral proteins are involved in dengue virus (DENV) replication. Heterogeneous ribonucleoprotein (hnRNP) C1/C2 are abundant host cellular proteins that exhibit RNA binding activity and play important roles in the replication of positive-strand RNA viruses such as poliovirus and hepatitis C virus. hnRNP C1/C2 have previously been shown to interact with vimentin and viral NS1 in DENV-infected cells; however, their functional role in DENV replication is not clearly understood.
Researchers investigated the role of hnRNP C1/C2 in DENV replication by using an in vitro model of DENV infection in a hepatocyte cell line (Huh7) and siRNA-mediated knockdown of hnRNP C1/C2. The results suggest that hnRNP C1/C2 is involved in DENV replication at the stage of viral RNA synthesis. These findings pave the way for further study on the molecular mechanisms of the viral and host protein interactions required for viral replication in DENV-infected cells.

This study was conducted by Chiang Mai University; Faculty of Medicine Siriraj Hospital, Mahidol University, Medical Biotechnology Research Laboratory and Biomedical Technology Research Laboratory.


Adaptor protein 1A facilitates dengue virus replication

Rearrangement of membrane structure induced by dengue virus (DENV) is essential for replication, and requires host cellular machinery. Adaptor protein complex (AP)-1 is a host component, which can be recruited to components required for membrane rearrangement. Therefore, dysfunction of AP-1 may affect membrane organization, thereby decreasing replication of virus in infected cells.

In the study, it was demonstrated that AP-1-dependent traffic inhibitor inhibited DENV protein expression and virion production. Researchers further clarified the role of AP-1A in the life cycle of DENV by RNA interference. AP-1A was not involved in DENV entry into cells. However, it facilitated DENV RNA replication. RNAi specific to AP-1A decreased viral RNA and protein levels, and virion production in Huh7 cells. Huh7 cells transfected with AP-1A siRNA showed greater modification of membrane structures and fewer vesicular packets compared with cells transfected with control siRNA. Therefore, AP-1A may partly control DENV-induced rearrangement of membrane structures required for viral replication.

This study was conducted by the Faculty of Medicine Siriraj Hospital, Mahidol University, National University of Singapore and Medical Biotechnology Research Laboratory.


Potential biomarker for prediction of severe dengue

Shedding of microparticles (MPs) is a consequence of apoptotic cell death and cellular activation. Low levels of circulating MPs in blood help maintain homeostasis, whereas increased MP generation is linked to many pathological conditions.

In this study, researchers investigated the role of MPs in dengue virus (DENV) infection. Infection of various susceptible cells by DENV led to apoptotic death and MP release. These MPs harbored a viral envelope protein and a nonstructural protein 1 (NS1) on their surfaces. Ex vivo analysis of clinical specimens from patients with infections of different degrees of severity at multiple time points revealed that MPs generated from erythrocytes and platelets are two major MP populations in the circulation of DENV-infected patients. Elevated levels of red blood cell-derived MPs (RMPs) directly correlated with DENV disease severity, whereas a significant decrease in platelet-derived MPs was associated with a bleeding tendency. Removal by mononuclear cells of complement-opsonized NS1-anti-NS1 immune complexes bound to erythrocytes via complement receptor type 1 triggered MP shedding in vitro, a process that could explain the increased levels of RMPs in severe dengue. These findings point to the multiple roles of MPs in dengue pathogenesis. They offer a potential novel biomarker candidate capable of differentiating dengue fever from the more serious dengue hemorrhagic fever.

This research was a collaborative work among scientists from Songkhla Hospital, Khon Kaen Hospital, Mahidol University and Medical Biotechnology Research Laboratory.

Highlights from Tuberculosis Research

Genotypic diversity of drug-resistant tuberculosis isolates in Thailand

Drug-resistant tuberculosis (TB), which includes multidrug-resistant (MDR-TB), quinolone-resistant (QR-TB) and extensively drug-resistant tuberculosis (XDR-TB), is a serious threat to TB control. To identify whether the emergence of drug-resistant TB is attributable to transmitted resistance or acquired resistance, researchers set to characterize the genotypic diversity of drug-resistant TB clinical isolates collected in Thailand. Spoligotyping, a simple and rapid method for detecting polymorphisms within the direct repeat locus, was used as a first-line genotyping tool. A 24-locus MLVA was used for further subtyping of drug-resistant *M. tuberculosis* isolates.

Results show the usefulness of genotypic diversity as a supportive tool for understanding the causes of emergence drug-resistant TB over the study period. Firstly, the clonal spread of MDR-TB and XDR-TB provided the genetic evidence of previous MDR-TB community outbreak and revealed a hidden spreading event of XDR-TB, respectively. Secondly, three major clusters among all resistance groups suggested the spread of predominant clones and amplification of resistance. Finally, a moderate number of unique genotypes hinted at the possibility of acquired resistance as another factor driving the emergence of drug-resistant TB. The results of this study emphasize the need to strengthen TB control strategies and to adopt appropriate treatment regimens to prevent the further development of drug resistance.

This work was a collaborative study by researchers from the Faculty of Medicine Siriraj Hospital, Mahidol University, the Research Institute of Tuberculosis (Japan) and Medical Molecular Biology Research Unit.


Rapid and low-cost detection platform for multidrug-resistant tuberculosis

The traditional culture based drug susceptibility testing (DST) is the primary diagnostic platform for multidrug-resistant tuberculosis (MDR-TB) in most developing countries. The consequent diagnostic time-delay is a major cause of escalating incidence. The key to preventing further spread is early detection and treatment. While several molecular tests exist, they are limited by complexity and cost, hindering their widespread application.

Researchers developed a simple Nucleic Acid Lateral Flow (NALF) immunoassay to complement conventional PCR, for the rapid molecular detection of MDR-TB. The NALF device was designed using antibodies for the indirect detection of labeled PCR amplification products. Multiplex PCR was optimized to permit the simultaneous detection of the drug resistant determining mutations in the 81-bp hot spot region of the *rpoB* gene (rifampicin resistance), while semi-nested PCR was optimized for the S315T mutation detection in the *katG* gene (isoniazid resistance). This newly designed NALF is a simple, rapid and low-cost device suitable for low resource settings where conventional PCR is already employed on a regular basis. Moreover, the use of antibody-based NALF to target primer-labels, without the requirement for DNA hybridization, renders the device generic, which could easily be adapted for the molecular diagnosis of other infectious and non-infectious diseases requiring nucleic acid detection.
This work was conducted by the Faculty of Medicine Siriraj Hospital, Mahidol University, National Nanotechnology Center and Medical Molecular Biology Research Unit.


Highlights from Energy and Environment

Modified reactor for treating raw palm oil mill effluent

The wastewater from palm oil production, commonly known as palm oil mill effluent (POME), typically contains high levels of free fat, oil and grease (FOG), and high concentrations of suspended solids and colloidal components, such as oil and biofibers, which pose problems in the operation of anaerobic processes for wastewater treatment and biogas production. Either physical treatment or chemical treatment was employed to remove fine suspended solids and FOG from POME before processing in high-rate anaerobic reactors. However, it is desirable to investigate the possibility of developing anaerobic reactors which can process raw POME without chemical or physical pretreatments, to keep the process simple and reduce time, cost and the use of chemicals.

In this study, a modified continuous stirred tank reactor (CSTR) with a deflector installed at its upper section to promote the retention of suspended solids in the reactor was used to treat raw POME to produce biogas. Neither physical pretreatment nor chemical pretreatment was performed to remove biofibers and FOG. This modified CSTR can be operated with raw POME at the highest organic loading rate (OLR) of 19.0 g COD/l d, producing methane at 4.14 l/l d. Both cellulose and hemicelluloses were degraded at significant rates in the modified CSTR.

This study was conducted by researchers from King Mongkut’s University of Technology North Bangkok, King Mongkut’s University of Technology Thonburi and Waste Utilization and Management Laboratory.


Enhancement of starch-pulp separation in cassava starch production

In the cassava starch production process, starch granules following rasping step are divided into free and bound starch; the latter remains in the pulp and is difficult to separate, while the former is not bound inside the pulp complex structure. In a starch extractor, cassava starch granules are separated from pulp through the mechanisms of centrifugation and filtration.

To obtain information on the starch granule characteristics and centrifugal-filtration process in cassava starch production, researchers investigated the effects of particle size and variety of cassava root, centrifugation and filtration mechanisms on free starch granule separation efficiency. Three cassava root varieties, Rayong 9, Rayong 11 and Kasetsart 50, were classified by particle size after grinding and sieving. Experiments were conducted at various relative centrifugal forces and pressure drops. The free starch separation efficiency increased with decreasing particle size of all cassava root varieties. The grinding of cassava root into small pieces caused cell wall breakage, facilitating free starch separation from the pulp. As the relative centrifugal force increased, some bound starch granules were released due to the force acting on the cassava pulp. The pressure drop in filtration process drove the free starch granules to pass through the screen although
this force was insufficient to separate the bound starch granules from the fiber. The results of this study will be useful for designing the improvement of starch extraction, thus promoting resource utilization efficiency in the starch factory.

This study was conducted by researchers from King Mongkut’s University of Technology Thonburi in collaboration with Waste Utilization and Management Laboratory.


**Highlights from Biodiversity Research and Utilization**

**Collection of biological materials**

BIOTEC established the BIOTEC Culture Collection (BCC) in 1996 to be a depositary and distribution center for microbes. The facility is also designated by the Department of Intellectual Property as a repository of patent-related microorganisms. At present, a total of 75,926 strains are preserved at the BCC, of which 63.44% are fungi, 26.77% are bacteria, 9.51% are yeast and 0.28% are algae. BCC on-line catalogue holds a total of 7,614 strains which are ready for distribution service to clients. All strains in BCC are maintained by freezing and/or freeze drying to ensure their genetic stability for long term storage. Precautionary steps have been taken to ensure the survival and integrity of the preserved cultures, including temperature control and regulator units, temperature monitoring with alert systems and back-up power-storage units. BCC has also been certified with ISO 9001:2008 as a service provider since 2005.

BIOTEC has also collected and preserved other biological materials under BIOTEC Molecular Genomic Materials Collection (BMGC) for both research and industrial utilization. A total of 216 biological materials are currently being preserved in the collection, comprising 45 vectors, 25 hosts and 146 recombinant clones. BIOTEC Bangkok Herbarium (BBH) preserves 39,860 dried specimens of fungi; whereas Hybridoma Bank has 644 monoclonal antibody-secreting hybridomas.
In February 2015, BIOTEC officially launched Thailand Bioresource Research Center (TBRC) with the main mission to promote accessibility and utilization of biological materials, operating within a new concept of a value co-creation and co-innovation approach through network interaction. TBRC aims to hold selected high quality biological materials with known characteristics for services to its clientele with an international quality standard “OECD best practice guidelines for biological resource center”. TBRC also forms partnerships with other culture collections locally and internationally, so that clients can access and place an order for microorganisms from partner collections through TBRC. At present, TBRC microbial catalogue contains 10,019 microbial strains from five collections (TBRC, BCC, Thailand Institute of Scientific and Technological Research (TISTR), Chiang Mai University and Khon Kaen University). These are categorized as 2,143 strains of bacteria, 3,509 strains of filamentous fungi and 4,367 yeast strains, among these 472 are type strains. Other types of biological materials such as antibody and plasmid will be available for service in 2016. TBRC is also a member of the World Federation for Culture Collections (WFCC).

**Microbial diversity study**

Microbial diversity study at BIOTEC involves the collection, isolation and identification of microorganisms from a variety of natural habitats. A total of fourteen novel species were discovered in 2015 and many of these discoveries were made in collaboration between researchers from BIOTEC and other academic and research institutes. They are:

- Three new insect-pathogenic fungal species: *Ophiocordyceps septa* (isolated from unidentified ants of the genus *Camponotus* in Thailand), *Ophiocordyceps rami* (isolated from unidentified ants of the genus *Camponotus* in Thailand) and *Aschersonia narathiwatensis* (isolated from whitefly nymphs (*Hemiptera*) collected at Hala-Bala Wildlife Sanctuary in Thailand).

- A new genus and species of an aero-aquatic helicosporous fungus: *Helicocentralis hyalina* gen. et sp. nov.

- Six new yeast species: *Yamadazyma insecticola* f.a., sp. nov. (isolated from frass of an unidentified insect), *Yamadazyma epiphylla* f.a., sp. nov. (isolated from the external surfaces of rice leaves), *Hannaella siamensis* sp. nov. (isolated from rice leaves), *Hannaella phetchabunensis* sp. nov. (isolated from corn leaf), *Occultifur tropicalis* f.a., sp. nov. (isolated from sugarcane) and *Hannaella phyllophila* sp. nov. (isolated from plant leaves collected in Thailand and Taiwan).

- Four new bacterial species: *Acetobacter thailandicus* sp. nov. (isolated from a flower of the blue trumpet vine), *Sinosporangium fuscum* sp. nov. (isolated from an evergreen forest soil sample), *Micromonospora fluostatini* sp. nov. (isolated from near-shore sediment in Thailand) and *Actinoplanes luteus* sp. nov. (isolated from dry evergreen forest soil collected in Thailand).

Two new species of *Ophiocordyceps unilateralis*: *Ophiocordyceps septa* (left) and *Ophiocordyceps rami* (right) on unidentified ants of the genus *Camponotus*.

A new species of insect-pathogenic fungi, *Aschersonia narathiwatensis* grown on whitefly nymphs (*Hemiptera*).
Bioactive compound discoveries

BIOTEC natural product discovery program aims to explore microbial resources for bioactive compounds that have potential for pharmaceutical and agricultural product development. Bioactive substances produced from various microorganisms are identified by activity-guided fractionation and subjected to spectroscopic analyses to determine chemical structures. In 2015, a total of 133 active compounds were identified, 40 of which were novel compounds. Below are examples of microbial-derived compounds that have been identified in 2015:

- Two new cyclic carbonate derivatives, aspergillusols A and B, one new eutypinic acid derivative, aspergillusic acid, two new phenalenones, aspergillusanones A and B, one new cytochalasin, aspergillusachalasin, and one new y-butyrolactone, aspergilluslactone, along with six known secondary metabolites were isolated from the soil fungus Aspergillus sp. PSU-RSPG185. Aspergillusols A and B contain an unusual cyclic-carbonate functionality; whereas aspergillusanones A exhibited weak activity toward KB and Vero cells.

- Four new compounds including two eremophilane sesquiterpenes, penicilleremophilanes A and B, as well as two sulfur-containing biphenols, penicillithiophenols A and B, were isolated from the soil fungus Penicillium coticola PSU-RSPG138. Their structures were elucidated by spectroscopic methods. Known sporogen AO-1 and penicilleremophilanes A exhibited antimalarial activity against Plasmodium falciparum.

- Two new hydroanthraquinones, paradictyoarthrins A and B, were isolated from the mangrove-derived fungus Paradictyoarthriniun diffractum BCC 8704. These compounds exhibited cytotoxic activities.

- Four new metabolites, including two meroterpenoids, an isocoumarin, and a phenol, were isolated from the seagrass-derived fungus Pestalotiposis sp. PSU-ES194 together with eight known compounds. The biosynthetic pathway for isolated meroterpenoids is proposed and employed to establish their absolute configurations. The isolated compounds were evaluated for antimicrobial, antymycobacterial, antimalarial and cytotoxic activities.

- One new hydronaphthalenone derivative was isolated from the broth extract of the endophytic fungus Daldinia escholtzii PSU-STD57, together with five known compounds. All the compounds were tested for antimicrobial activity against Staphylococcus aureus, methicillin-resistant S. aureus and Microsporum gypseum.

- Six new compounds, an N-hydroxy pyridone glucoside, orbiocrellin A, its aglycone orbiocrellin B, chromone glucosides, a dihydrochromone and a chromone, were isolated from the scale-insect pathogenic fungus Orbiocrella sp. BCC 33248. Orbiocrellin A exhibited antimalarial activity against Plasmodium falciparum K1 while it was non-cytotoxic. In contrast, orbiocrellin B showed both antimalarial and cytotoxic activities.

- Two new ascochlorin derivatives, nectchlorins A and B, together with eight known compounds, were isolated from cultures of the leafhopper pathogen Microcera sp. BCC 17074. Cytotoxic activities of these ascochlorin derivatives were evaluated.
Four new compounds, which were (−)-(7R)-7-O-methylsydonic acid, gibellulic acid, gibellulins A and B, together with 14 known compounds were isolated from the insect pathogenic fungus, Gibellula sp. BCC36964. These compounds were evaluated for the biological activities including anti-bacterial, anti-phytopathogenic, anti-Herpes simplex virus-type 1 activities, and cytotoxicity against both cancerous and non-cancerous cells.

Four quinazolinones, new naturally occurring and three known derivatives, along with previously synthesized 4-phenylbut-3-enamide and three known compounds were isolated from an actinomycete, Streptomyces sp. BCC 21795. The new compound exhibited strong cytotoxic activity to Vero cells.

Two new prenylhydroquinone-derived compounds, lentinospirol and 1-(2,5-dihydroxyphenyl)-4-hydroxy-3-methyl-l-butanone, were isolated from cultures of the basidiomycete Lentinus similis BCC 52578, together with five known compounds.

A new naphthoquinone, solaninaphthoquione, and a new succinate ester derivative, 4-(4-hydroxyphenethoxy)-4-oxobutanoic acid, were isolated from the soil fungus Fusarium solani PSU-RSPG227 together with five previously reported compounds. Solaninaphthoquione showed significant cytotoxic activity against breast cancer (MCF-7) cells, mild cytotoxic activity against oral human carcinoma (KB) cells and weak antimalarial activity.

Twelve aromadendrane sesquiterpenoids, inonotins A–L, and a previously unknown cyclofarnesane, i.e., inonofarnesane, together with two known compounds, were isolated from cultures of the wood-rotting basidiomycete Inonotus sp. BCC 23706.

**Insights to pathogenicity and host specificity in insect fungi**

Ophiocordyceps unilateralis is referred to as a zombie ant fungus because it causes the infected host ant to climb into vegetation, bite vegetal materials then hang themselves upside down until death. The biology of O. unilateralis still has much to be discovered and the genome sequencing of this species will help gain an insight.

BIOTEC researchers reported a draft genome of Ophiocordyceps polyrhachis-furcata, a species in the O. unilateralis species complex, which is also a Hypocreaealan entomopathogenic fungus. Comparative analyses on genes involved in pathogenicity and virulence between O. polyrhachis-furcata and other fungi were performed. The research team identified genes involved in various steps of pathogenesis, investigated the common attributes of being entomopathogenic and the extent to which the host ranges have shaped their evolution as well as enabling the discovery of new biosynthetic pathways.

This study was a collaborative effort between Bioresources Technology Research Unit and Genomic Technology Research Unit.

Microbial diversity in flood areas during Thailand's 2011 flood

In late 2011, Thailand experienced one of the most damaging floods of the century. Flooding spread through the provinces of Northern, Northeastern and Central Thailand along the Mekong and Chao Phraya river basins and lasted for months in some areas, especially in the Central Plain. The flood ecosystem is considered a temporarily variable ecological-niche within the aquatic environment that can have a major impact on microbial communities related to water quality and public health.

In this study, bacterial and fungal diversity in sediments and waters collected from ten flood areas in Bangkok and its suburbs, covering residential and agricultural areas, were analyzed using high-throughput 454 pyrosequencing of 16S rRNA gene and internal transcribed spacer sequences. Analysis of the microbial community showed differences in taxa distribution in water and sediment with variations in the diversity of saprophytic microbes and sulfate/nitrate reducers among sampling locations, suggesting differences in microbial activity in the habitats. Overall, Proteobacteria represented a major bacterial group in waters, while this group co-existed with Firmicutes, Bacteroidetes, and Actinobacteria in sediments. Analysis of the microbial community showed differences in taxa distribution in water and sediment with variations in the diversity of saprophytic microbes and sulfate/nitrate reducers among sampling locations, suggesting differences in microbial activity in the habitats. Overall, Proteobacteria represented a major bacterial group in waters, while this group co-existed with Firmicutes, Bacteroidetes, and Actinobacteria in sediments. Anaeromyxobacter, Steroidobacter, and Geobacter were the dominant bacterial genera in sediments, while Sulfuricurvum, Thiovirga, and Hydrogenophaga predominated in waters. For fungi in sediments, Ascomycota, Glomeromycota, and Basidiomycota, particularly in genera Phylipis, Rosella, and Acaulospora, were most frequently detected. Chytridiomycota and Ascomycota were the major fungal phyla, and Rhizophlyctis and Mortierella were the most frequently detected fungal genera in water. Diversity of sulfate-reducing bacteria, related to odor problems, was further investigated using analysis of the dsrB gene which indicated the presence of sulfate-reducing bacteria of families Desulfobacteraceae, Desulfbulbaceae, Syntrobacteraceae and Desulfoarcaceae in the flood sediments. The work provides a foundation for more detailed studies on how microbial communities in flood ecosystems affect physicochemical changes in the environment and impact human communities.

This study was the result of collaborative work by researchers from Chulalongkorn University, North Dakota State University (USA) and BIOTEC.

Study of polyketide synthase (PKS) genes in entomopathogenic fungi

Entomopathogenic fungi are able to invade and kill insects. Various secondary metabolites can mediate the interaction of a fungal pathogen with an insect host and also help the fungus compete with other microbes.

Researchers screened 23 isolates of entomopathogenic fungi for polyketide synthase (PKS) genes and amplified 72 PKS gene fragments using degenerate PCR. A phylogenetic analysis was performed and 72 PKSs were identified from four insect fungal genome sequences. The resulting genealogy indicated 47 orthologous groups with 99–100% bootstrap support, suggesting sharedbiosynthesis of identical or closely related compounds from different fungi. Three insect-specific groups were identified among the PKSs in reducing clades IIA, IIB, and III, which comprised PKSs from 12, 9, and 30 fungal isolates, respectively. A IIA-IIB pair could be found in seven fungi. Expression analyses revealed that eleven out of twelve PKS genes identified in Beauveria bassiana BCC 2660 were expressed in culture. PKS genes from insect-specific clades IIA and IIB were expressed only in insect-containing medium, while others were expressed only in PDB or in CYB, PDB and SDY. The data suggest the potential production of several polyketides in culture. As fungal polyketides from these entomopathogens represent valuable natural product resources, these PKS genes’ phylogeny and expression data would be important for further studies of valuable polyketides in the future.

This study was performed by Bioresources Technology Research Unit in collaboration with King Mongkut’s University of Technology Thonburi and Assumption University.

Role of tenellin in iron homeostasis in *Beauveria bassiana*

Iron is an essential element for life. However, iron overload can be toxic. In this study, researchers investigated the significant increase of tenellin and iron-tenellin complex production in ferricrocin-deficient mutants of *Beauveria bassiana*. The chemical analysis indicated that the ferricrocin-deficient mutants nearly abolished ferricrocin production. In turn, these mutants had significant accumulation of iron-tenellin complex in their mycelia under iron-replete condition. Both tenellin and iron-tenellin complex were not detected in the wild-type under such condition. Mass analysis of the mutants’ crude extracts demonstrated that tenellin formed a 3:1 complex with iron in the absence of ferricrocin. The unexpected link between ferricrocin and tenellin biosynthesis in ferricrocin-deficient mutants could be a survival strategy during iron-mediated oxidative stress.

This study was conducted by Bioresources Technology Research Unit and King Mongkut’s University of Technology Thonburi.


**Powerful tool for engineering polyunsaturated fatty acid biosynthesis**

$\Delta^6$-Desaturase is a key enzyme involved in the biosynthesis of polyunsaturated fatty acids (PUFAs). To manipulate the oil composition of organisms of choice, by a metabolic engineering approach, a high expression level of the $\Delta^6$-desaturase enzyme in a heterologous host is required. As well as optimized culture conditions, an efficient promoter and substrate availability, codon optimization is one of the most common approaches for improving heterologous gene expression.

Researchers cloned a $\Delta^6$-desaturase gene from *Pythium* sp. The gene encoded an enzyme which catalyzed the $\Delta^6$-desaturation of the fatty acyl substrates having $\Delta^9$-double bond. The product yields were markedly enhanced by codon optimization of the *Pythium* gene. This codon-optimized $\Delta^6$-desaturase gene is a promising tool for further reconstitution of the fatty acid profile, in a host system of choice, for the production of economically important fatty acids, particularly the $n$-3 and $n$-6 polyunsaturated fatty acids.

This work was conducted by Bioresources Technology Research Unit.


**Highlights from Biorefinery**

**Binding characteristic and synergistic effect of expansins on cellulose degradation**

Degradation of lignocellulosic plant biomass is a key bio-geochemical process in the organic carbon cycle. Expansins are non-hydrolytic proteins which have been demonstrated to enhance the hydrolytic efficiency of lignocellulose by loosening the plant cell wall structure. The molecular mechanism of how expansins enhance cellulose degradation is unclear and limited study has been performed on their substrate binding characteristics and interaction with cellulase-degrading enzymes.

Researchers identified the binding characteristics of five expansins originating from different groups of bacteria on cellulosic and hemicellulosic polysaccharides. The synergistic action of these expansins with *Trichoderma reesei* cellulase on different types of polysaccharides was also studied. The work provides an insight into the biological functions of expansins on plant cell wall degradation with the potential uses on enhancing degradation and modification of plant lignocellulosic polysaccharides in bio-industries.

This study was performed by Bioresources Technology Research Unit in collaboration with Kasetsart University.

Modified Clean Fractionation method

Fractionation of lignocellulosic constituents is a prerequisite for efficient utilization of plant biomass in integrated biorefineries. Several techniques have been developed for biomass fractionation, including a newly-reported method termed Clean Fractionation (CF).

In this study, researchers tested different organic solvents and acid promoters for their effects on fractionation of rice straw using a modified CF process.

Ethyl acetate showed comparable efficiency in separation of biomass components compared with methyl isobutyl ketone (MIBK) as used in the conventional process. In addition, microwave treatment was used to replace conventional heating, with the advantages of lower formation of inhibitory by-products. The work provides an efficient alternative approach for separation of primary lignocellulosic components with high recoverability and selectivity for further valorization in integrated biorefineries.

This study was performed by Bioresources Technology Research Unit, King Mongkut’s University of Technology Thonburi, PTT Research and Technology Institute and PTT Group Frontier Research Center.


Highlights from Bioinformatics

SpirPro: Spirulina-Proteome Repository

Cyanobacteria have been experimentally used as a model for plants and bacteria, therefore many cyanobacteria, such as Synechocystis sp., Synechococcus sp., and Spirulina (Arthrospira) platensis, have been studied at the genomic and proteomic levels.

In the quest to understand the effect of fluctuating environmental temperatures on Spirulina mass cultivation, researchers performed proteomic analyses of Spirulina under optimal and temperature stress conditions. The study led to the development of SpirPro, an integrated database for Spirulina. This database provides possible mechanisms, in terms of protein-protein interaction networks and proteome-wide expression levels, underlying the temperature stress response of this cyanobacterium for use as model mechanisms for other photosynthetic organisms, such as plants, algae and other cyanobacteria. Moreover, proteome-wide domain identification is available in the database, which might be useful for further studies on protein-protein interaction domain analyses. SpirPro is publicly available on-line at http://spirpro.sbi.kmutt.ac.th.

This work was a joint study between Biochemical Engineering and Pilot Plant Research and Development
Laboratory and King Mongkut’s University of Technology Thonburi.


**Comprehensive database for human–mouse comparative study of microRNA–promoter interactions**

In 2012, microRNA (miRNA)–promoter interaction resource (microPIR) was introduced as a comprehensive public database containing over 15 million predicted miRNA target sites located within human promoter sequences. The database assists experimental scientists in exploring interactions of interest and facilitates research in this area. Since the release, access to the predicted targets in the database has markedly increased, demonstrating the potentially increasing interest in the research of this topic and the potential value of the microPIR database in the future.

Given the conservation of miRNAs and associated gene regulatory mechanisms across species, it is plausible that the regulatory role of miRNA through promoter recognition is also present among mammalian species. Researchers therefore made modifications and updated the database, naming it microPIR2, which contains predicted miRNA promoter targets in the mouse genome. microPIR2 provides approximately 80 million human and 40 million mouse predicted target sites. In addition to being a reference database, microPIR2 is a tool for comparative analysis of target sites on the promoters of human–mouse orthologous genes. In particular, this new feature was designed to identify potential miRNA–promoter interactions conserved between species that could be stronger candidates for further experimental validation. Additional supporting information such as nuclear and cytoplasmic localization of miRNAs and miRNA–disease association is also incorporated into this new database.

microPIR2 was developed by Biostatistics and Bioinformatics Laboratory. The database is available on this website: http://www4a.biotec.or.th/micropir2.


**Highlights from Diagnostic Technology**

**Bead array for *Listeria* detection**

Bacteria of the genus *Listeria* currently contain six species, but only *L. monocytogenes* is pathogenic to humans, causing listeriosis. Well established traditional methods are tedious and time consuming and thus are not suitable for screening large numbers of food samples required to meet increasing global demand. Some high-throughput methods based on detecting nucleotide sequences need sophisticated detectors, elaborate sample extraction, and highly-trained personnel. Therefore, immunoassays are still preferred by many end-users for high volume sample screening.

Bead array for *L. monocytogenes* detection has been developed using specific monoclonal antibodies. The novel highly specific antibodies were obtained from hybridoma libraries generated by using formalin-killed and heat-killed *L. monocytogenes* as immunogens. The bead array was able to detect the bacteria with the same accuracy as the standard plating method at the 1 CFU level after only 24 h of the enrichment period. In addition, *Listeria*-specific 3C3 and *L. monocytogenes*-specific 7G4 antibodies were successfully employed to construct a multiplex detection for *Listeria*, *Salmonella* and *Campylobacter* in a bead array format by combining with commercial *Salmonella*-specific and available *Campylobacter*-specific antibodies.
This study was a collaborative work between Biosensing Technology Research Unit, Animal Biotechnology Research Unit and Queen’s University Belfast (UK).


Bovine embryo sex determination by multiplex loop-mediated isothermal amplification

The ability to determine the sex of cattle embryos before transfer is useful for livestock management, particularly in the dairy cattle industry where female calves are preferred. The current methods for sexing bovine embryos normally rely on detecting Y chromosome–specific DNA using PCR or fluorescence in situ hybridization (FISH). However, these techniques are not widely used in the field as they are time consuming and labor intensive and require expensive instruments. Alternatively, a loop-mediated isothermal amplification (LAMP) technique can be used. However, two separate LAMP reactions, one for the male-specific detection and the other for the control reaction, are required for complete bovine sexing.

Researchers developed a modified LAMP in a multiplex format (multiplex LAMP) for highly efficient bovine embryo sexing. The protocol can simultaneously detect both male-specific DNA and control DNA in a single reaction tube using multiplex LAMP. Two chromosomal regions, one specific for males (Y chromosome, S4 region) and the other common to both males and females (1.715 satellite DNA), were amplified in the same reaction tube. Each target was amplified by specifically designed inner primers, outer primers and loop primers, where one of the S4 loop primers was labeled with the fluorescent dye 6-carboxyl-X-rhodamine (emitting a red color), whereas both satellite loop primers were labeled with the fluorescent dye fluorescein isothiocyanate (emitting a green color). After amplification at 63°C for 1 hour, the amplified products were precipitated by a small volume of cationic polymer predispensed inside the reaction tube cap. Green precipitate indicated the presence of only control DNA without the Y chromosome, whereas orange precipitate indicated the presence of both target DNAs, enabling interpretation as female and male, respectively. The multiplex LAMP showed 100% accuracy in identifying the actual sex of the embryos. The method is cost effective and highly mobile, making it suitable for field use.

This study was conducted by Animal Biotechnology Research Unit, Food Biotechnology Research Unit, Chiang Mai University and Suranaree University of Technology.


New method for white spot syndrome virus detection using graphene oxide

Graphene oxide (GO) is attractive for biological or medical applications due to its unique electrical, physical, optical and biological properties. In particular, its fluorescence quenching property has been exploited for biosensing via fluorescence resonance energy transfer (FRET) mechanism.

Researchers developed a white spot syndrome virus (WSSV) DNA detection method based on loop-mediated isothermal amplification (LAMP) combined with FRET between GO and fluorescein isothiocyanate-labeled probe (FITC-probe). The technique has a detection limit of 10 copies of WSSV plasmid DNA or 0.6 fg of DNA extracted from shrimp infected with WSSV and no cross contamination.

The results demonstrated that GO-FRET with LAMP technique is promising for fast, sensitive and specific DNA detection and could be applied to a point-of-operating system.
This study was conducted by Biosensing Technology Research Unit and National Electronics and Computer Technology Center.


**Highlights from Policy Research**

**Study on the potential of hybrid rice technology in Thailand**

It is well documented that hybrid rice varieties yield about 15 to 20 percent more than even the best of the improved or high-yielding bred varieties do. Moreover, hybrid technology expedites breeding several desired traits into one variety. Hybrid rice technology has been well exploited in China, and many rice-producing countries are now turning their attentions to hybrid rice study in recognition of its good potential to improve food security and competitiveness. Hybrid rice however, is not well adopted by farmers in Thailand because of the poor performance of imported hybrid seeds under the local climate and conditions and the high price of domestic seed due to the lack of efficient hybrid seed production technology. Despite putting continuous effort into rice breeding, yield growth of Thai rice is declining, indicating the limitation of pure line cultivars. The question remains whether Thailand should pay more attention to this technology.

BIOTEC research team conducted a study on the potential of hybrid rice technology in Thailand focusing on three aspects: 1) market opportunity 2) farmers’ adoption and 3) technological capability. The study revealed that common white rice, among several types of rice grown in Thailand, has potential for employing hybrid seed technology, as it is cultivated for commercial purpose and has various industrial applications in the value chain analysis, thus yield improvement through hybrid technology will help growers and industries gain substantial advantage. Farmers will likely adopt the technology if the hybrid line performs far better than pure lines. To promote its technological capability in hybrid rice, an R&D and capacity building plan needs to be in place, along with the plan to promote hybrid rice industry.

The report of this study was submitted to Thailand Research Organizations Network, a network of seven government research funding agencies.

**Technology Action Plans for the agricultural sector to adapt to climate change**

Agriculture remains a fundamental part of the Thai economy and social structure. For Thailand, climate change could therefore have significant effect on the agricultural sector, far more than any other sectors. Three technologies have been identified as crucial technologies in defining how Thailand can respond to, and mitigate the effects of, climate change on the agricultural sector. They are: 1) forecasting and early warning systems, 2) crop improvement and 3) precision farming. Subsequently, Technology Action Plans (TAPs) were formulated for the next ten years, 2015-2025. TAPs comprise two sections: 1) Technology Action Plans for building research capability and human resources, including priority crops and model crops, for each technology, and 2) Action Plans for Technology Transfer which address the cooperation among government agencies, academic sector, private sector and farmers and the funding to support learning centers at the district level to deliver training modules for government officers (smart officers) and farmers (smart farmers) and manage knowledge; all of which go to ensure that the technologies are adopted and exploited.

TAPs have been submitted to the National Science Technology and Innovation Policy Office, and have been incorporated into the Thailand Climate Change Master Plan 2015-2050 (led by Ministry of National Resources and Environment) and The Twelve National Agriculture and Cooperatives Plan 2017-2021 (led by Ministry of Agriculture and Cooperatives). Thailand Research Organizations Network, comprising seven government research funding agencies, has included climate change as a new target research initiative.
BIOTEC places strong emphasis on exploiting our research through the transfer of technologies to public or private sectors. The main mechanisms used to implement the technology transfer include technology and product licensing, capacity building, along with collaborative and commissioned research, as well as consultancy service.
Licensing Agreements

The following technologies were licensed during fiscal year 2015:

**Production technology for low-cyanide cassava flour.** The technology is able to process bitter cassava, known to contain high levels of toxic cyanide, into high quality cassava flour with less than 10 ppm cyanide content. This technology can be installed in the cassava starch factory with some process modification, enabling the factory to diversify its products. Chorchaiwat Industry Company Limited, a starch-processing company, obtained the license to use this technology to produce cassava flour for market testing.

**Colloidal solution as an indicator for acetone gas.** This technology involves two types of reagents which when mixed will react specifically with acetone gas resulting in a color change. The detection range of acetone is between 0 to 100 ppm. Health Innovision Company Limited has licensed the technology in order to further develop it into a breath analyzer for non-invasive glucose monitoring.

The licenses for the following technologies were extended during fiscal year 2015:

**Air filter for tissue culture laboratory.** An air filter for greenhouses or tissue culture containers, developed by BIOTEC researchers, creates an environmental condition that allows for air and moisture exchange in the tissue culture process, keeping the moisture content within the tissue culture container at 45-65%, an optimal level for plant growth. The technology was licensed to a tissue culture business operator in 2008 and the agreement was renewed with the same operator in 2015.

**Alpha thalassemia immunochromatographic strip test.** The immunochromatographic strip is a qualitative, lateral flow immunoassay for the screening of various types of alpha thalassemia traits by using whole blood. The test strip includes antibodies specific to the gamma 4 protein and provides easy visual discrimination between a positive result and a negative result. The technology was licensed to i+MED Laboratories Company Limited in 2011 and the agreement was renewed in 2015.
Collaborative and Commissioned Research

In fiscal year 2015, BIOTEC conducted 72 projects in the form of collaborative and commissioned research, as well as consultancy services. Of these, 29 projects were initiated in 2015. Collaboration and services covered a range of topics, such as screening for microorganisms and enzymes for specific purposes and the development of an industrial-scale production system, designing fermentation processes for food and feed products, breeding of commercial crops, improving wastewater treatment/biogas system, recovering value-added products from waste streams and diagnostic development for environmental and agricultural applications. Following are some of the highlights:

Establishment of standard protocol for disease-resistance screening in tomato with the Asia & Pacific Seed Association. The Asia & Pacific Seed Association (APSA) is the largest regional seed association in the world, whose aim is to promote quality seed production and marketing in the Asia and Pacific Region. BIOTEC collaborates with APSA to develop an efficient inoculation protocol for screening Tomato necrotic ringspot virus (TNRV) and Capsicum chlorosis virus (CaCV), two major tospovirus species causing severe damage to tomato plantations. The developed protocol will greatly enhance the development of disease-resistant tomato lines in the seed industry.

Integrative sugarcane breeding to increase sugar content with Mitr Phol Innovation and Research Center. Mitr Phol Group is Thailand’s and Asia’s biggest sugar and bio-energy producer. The research partnership brings in complimentary expertise from each party, namely BIOTEC’s advanced omic technologies and Mitr Phol’s competence in sugarcane breeding, to develop sugarcane elite lines with high sugar content, high yield, drought tolerance, longer ratooning and good genetic background. The results of this project will enable the sugar industry to overcome shortage of supply and inconsistency of sugar content from different cane varieties and locations throughout the year.

Screening for potential microorganisms for animal feed probiotics for Asia Star Trade. Asia Star Trade is a local company with core business in animal and aquaculture feed production. The company commissioned BIOTEC to find microorganisms with probiotic potential in order to develop these into a commercial feed supplement. Asia Star Trade already has a few feed enzyme products based on technologies developed by BIOTEC.

Developing formulations for commercial biocontrol products of Beauveria bassiana for Ladda. Ladda is a leading agro-chemical company in Thailand. The company commissioned BIOTEC to develop formulations for commercial biocontrol products based on one Beauveria bassiana strain screened by BIOTEC, which has been shown to exhibit efficacy against insect pests including green peach aphid (Myzus persicae), pink mealybug (Phenacoccus manihoti) and brown planthopper (Nilaparvata lugens).

Tomato necrotic ringspot virus (TNRV) and Capsicum chlorosis virus (CaCV) are the major tospovirus species detected in tomato.
Open Lab for Industry

Open Lab is an activity to promote collaboration between laboratories and industries by inviting companies to meet with BIOTEC scientists, learn about BIOTEC expertise and the potential of biotechnology for improving industrial processes and products. Open Lab often leads to collaborative research, commissioned research, or the provision of analytical services. In 2015, a total of 6 open lab events were organized. Held during the NSTDA Annual Conference (NAC), NSTDA Open House offered opportunities for the private sector to visit various laboratories located in Thailand Science Park, including BIOTEC laboratories. Over two hundred companies -- in various industries such as food and health food, agriculture, IT, chemicals and energy -- participated in NSTDA Open House. In addition, throughout the year, two open lab events were dedicated to agriculture and food industry with a total of 40 companies participating. One event was designed for 10 aquaculture companies with the showcase of various technologies to support aquaculture industry such as diagnostic technology for aquatic animals and recirculating aquaculture system.

Production technology for high-quality Beauveria for Thai farmers

To utilize Thailand natural resources for pest control, BIOTEC research team explores insect pathogenic fungi, which are natural enemies of pest insects, to discover potential strains that could become effective biological control agents that pose minimal threat to non-target organisms. From our studies, Beauveria bassiana BCC2660 has been shown to exhibit efficacy against insect pests including green peach aphid (Myzus persicae), mealybug (Pseudococcus cryptus) and brown planthopper (Nilaparvata lugens). Researchers then developed a solid-state fermentation process using rice grain as a substrate for the effective production of Beauveria inoculum at low cost. The process and conditions have been optimized to yield maximum spore production.

Realizing that one of the tasks undertaken by the Department of Agricultural Extension (DOAE) is to supply Beauveria inoculum to farmers to promote the use of biocontrol agents, NSTDA is collaborating with DOAE aiming to build up the capacity in Beauveria production in the Department, as well as with farmers. This will be achieved by the transfer of technology developed and the effective strain (B. bassiana BCC2660) screened by BIOTEC to regional centers of DOAE responsible for inoculum production and distribution, as well as the training of farmers on Beauveria production from inoculum. The pilot project will focus on upgrading production facilities and training of staff of DOAE regional centers in Chiang Mai, Phitsanulok, Phrae and Nakhon Sawan provinces, as well as farmers in those respective areas. It is expected that these four sites will further transfer the technology to other centers to cover the whole country.

The pilot project was launched in Chiang Mai on 22 June 2015, with a meeting among staff from NSTDA, BIOTEC and DOAE to formulate a detailed action plan. In addition, a training workshop on Beauveria inoculum production was held for DOAE staff on the same day.
Transfer of starter culture technology for silage production for cattle farms

In response to increasing demand for animal feed in the livestock sector, significant progress has been made in the improvement of forage crops in Thailand. Fast-growing and high-yield varieties of forage cane and napier grass have already been developed by BIOTEC-Kasetsart University and the Department of Livestock Development, respectively. However, feed shortage still persists during the dry season. To solve this problem, researchers from BIOTEC, Khon Kaen University and the Department of Livestock Development co-developed starter culture technology for ensilaging green fodder. Fast-growing lactic acid bacteria (LAB) were screened locally from the natural environment and used as starter culture to ensilage forage cane and napier grass when forages are high in quantity. It was found that, not only could the starter culture speed up the fermentation process, the silage produced from starter culture technology is of higher quality and can be preserved for a longer period of time compared to naturally-fermented silage. This technology thus enables farmers to stockpile high-quality feed into the dry season.

This technology has been transferred to livestock farmers and feed producers at the 2015 National Dairy Fair. The workshop was also organized in Chiang Mai province to transfer the technology to farmers and operators in the northern region.

blueAmp, a simple and rapid detection test for streptococcosis in Nile tilapia, was introduced to potential investors at NSTDA Investors’ Day 2015.
BIOTEC places a high priority on capacity building through increasing the quantity and quality of human resources in biotechnology as well as upgrading and educating the workforce. Several activities were designed to assist different segments of the workforce, as well as address a variety of objectives, ranging from providing fellowships, to training post-graduate students, to organizing scientific conferences and training workshops for academics and industry, as well as organizing youth programs.
In 2015, BIOTEC granted 11 post-doctoral fellowships, 3 of which are new fellowships. A total of 21 scientific events, including one international conference and twenty training workshops and seminars, were organized on various topics, such as plant breeding, microbial management, animal vaccine technology and guidelines for biosafety of genetically modified microorganisms. Science education was also promoted through the Science in Rural Schools (SiRS) program. In addition, BIOTEC also runs a program to build up capacity for farmers and community enterprises, offering training and technical assistance in Good Agricultural Practice and Good Hygiene Practice.
International Conference on Anaerobic Digestion

The International Conference on Anaerobic Digestion: AD Technology and Microbial Ecology for Sustainable Development (ADTech 2015) was held on 3-6 February 2015 in Chiang Mai, Thailand. The Conference was organized by a joint effort of the Institut National de la Recherche Agronomique (INRA), France, Chiang Mai University, King Mongkut’s University of Technology Thonburi and BIOTEC.

ADTech 2015 provided a primary platform for in-depth presentation and discussion on various topics that cover all aspects of anaerobic digestion namely microbial ecology in anaerobic environment; process engineering in anaerobic digestion; new biomass feedstock for renewable energy; methane emission and climate change; biogas utilization and upgrading; and policy-economic aspects of anaerobic digestion. The Conference program consisted of 58 lectures and 26 poster presentations. Keynote lecturers included Dr. Lodewijk Willem Hulshoff Pol (Lettinga Associates Foundation, the Netherlands), Prof. Théodore Bouchez (National Research Institute of Science and Technology for Environment and Agriculture, France), Prof. habil. Bernd Bilitewski (INTECUS GmbH Waste Management and Environment-Integrating Management, Germany), Prof. Makarand Madhao Ghangrekar (Indian Institute of Technology Kharagpur, India), Prof. Saulius Vasarevicius (Vilnius Gediminas Technical University, Lithuania) and Prof. Morakot Tanticharoen (King Mongkut’s University of Technology Thonburi, Thailand).

Poster awards were announced on the last day of the Conference. The awards went to Prof. Kazuaki Syutsubo (National Institute for Environmental Studies, Japan), Mr. Peerawat Khongkliang (Taksin University, Thailand) and Asst. Prof. Piyanuch Niamsup (Maejo University, Thailand) for gold, silver and bronze prizes, respectively.

A visit to the Landfill Gas Energy Site was offered to participants as part of the excursion. ADTech 2015 was well attended by 120 participants from 16 countries.
Building Up National Capacity in Plant Breeding

It has been reported that climate change will have a great effect on agricultural sector. As Thailand is an agriculture-based economy, with half of the land and 35% of labor force devoted to agriculture, this is a major threat to its economy and food security. To cope with the effect of climate change on food security, three technologies have been identified as crucial technologies for the development in the next 10 years: plant breeding; precision farming; and forecasting and warning systems.

BIOTEC and NSTDA, in collaboration with several Thai universities, have developed an intensive capacity building program in plant breeding in order to enhance human resource development in this particular area. The Program consists of 6 training modules to be offered through a series of workshops, namely:

- Module I: Breeding of Cucurbit
- Module II: Technology Tools for Breeding: Corn Model
- Module III: Learning from Success Case of Rice Marker-assisted Selection Breeding
- Module IV: Learning from Success Case of Fruit Tree
- Module V: Plant Genomics / Molecular Tools
- Module VI: Crop Breeding Concept and Success Case

Two training modules have been delivered in fiscal year 2015 while the rest will be offered in 2016.

Capacity Building in Biosafety of Genetically Modified Microorganisms

A genetically modified microorganism (GMM) is a microorganism whose genetic material has been altered using genetic modification techniques to produce desired genetic features for specific needs. In an industrial scale, GMMs have been utilized to produce specially designed products in a wide range of industries, from pharmaceutical and medical supply industry, food industry, to bio-industry. To ensure the human and environmental safety, the Technical Biosafety Committee (TBC) first released “Biosafety Guidelines for Contained Use of Genetically Modified Microorganisms at Pilot and Industrial Scales” back in 2004, with regular revisions for the most up-to-date information. The Guidelines cover the use of GMMs in containment at pilot and industrial scales according to GMM classification, together with suggested containment levels, GMM waste management, transport, possession, emergency plans and the responsibilities of personnel associated with GMM work.

Since the use of GMM has been on the rise, the understanding and awareness among practitioners is necessary for the proper handling. BIOTEC, in collaboration with Faculty of Science, Chulalongkorn University and Faculty of Science, Mahidol University, organized the Law and Regulation Workshop on How to Use Genetically Modified Microorganisms (GMMs) in Thailand on July 9, 2015, specifically targeting at regulatory officers and staff in the industry working with GMM. The Workshop was attended by 64 participants from 16 governmental organizations and 11 companies in Thailand.
Training of Science Teachers in the Rural Area

BIOTEC initiated the Science in Rural Schools (SiRS) program in 1998. The establishment followed the initiative of HRH Princess Maha Chakri Siridhorn to improve the quality of life and enhance learning capability in science for rural students and science teachers, through activities such as producing educational media such as books, a website and CD-ROM, as well as supporting schools to organize science camps and science project contests to stimulate science learning.

Since 2009, SiRS has taken part in a larger initiative, the Little Scientists’ House Thailand, aiming to promote science education among children by offering training and learning materials to science educators and creating a community of practice among science teachers at the provincial, regional and national levels through the collaboration with the Haus der kleinen Forscher Foundation of Germany. SiRS has been tasked to disseminate the learning materials developed by Little Scientists’ House Thailand to schools in its network in four provinces (Maehongson, Sakon Nakon, Pangnga and Narathiwat), by developing training modules for teachers in rural schools to use the learning materials to fit with the context of the area and providing training to school teachers on how to use the learning media in the classroom. Workshops and follow-up meetings were organized with the pilot schools to monitor the improvement in students’ learning abilities. In 2015, 109 teachers participated in the training program.
One of BIOTEC’s missions is to raise public awareness of how biotechnology and the life sciences relate to everyday life. This is part of the larger goal of making Thailand a knowledge-based economy. The mission is carried out in part by channeling information through popular media such as the internet and television, as well as providing information to journalists. BIOTEC also organizes exhibitions at various events, ranging from scientific conferences, as well as science and technology, agricultural and industry fairs. Lab tours are also organized to bring the public into BIOTEC research facilities where they can meet and talk to scientific staff.
Television Programs

In 2015, BIOTEC produced one television program, a 1.5 minute spot, for regular broadcast on public channels as part of NSTDA TV. A total of 19 shows were produced and went on air, as well as being made available for viewing on BIOTEC YouTube (http://www.youtube.com/biotecthailand). These television programs serve several purposes, from raising awareness of science, educating the public about the benefit of technologies developed locally, showcasing successful inventions that have reached the market and introducing products and technologies to potential investors or collaborators.

Press Activities

Events have been organized regularly for local journalists to be informed of our scientific research and the use of our technologies in actual industries. In 2015, members of the press were invited to visit:

- Thanapaisal, a textile factory that uses ENZease, a duo-activity enzyme for one-step biodesizing and bioscouring process of cotton fabrics;
- Rai Khunnatham, a grape producer that uses NPV to control insect pests in the vineyard;
- Organic rice farms in Yasothon province, where BIOTEC promotes and demonstrates the use of technologies to upgrade farming practices.

News and articles on these stories were subsequently published reaching out to the general public.
Exhibitions

BIOTEC participated in a number of exhibitions and displays at major events, disseminating results and ideas to several demographics and industrial sectors. Examples of key events were the 2015 National Dairy Day in January; IP Fair 2015 in February; NSTDA Annual Conference in April; Thai Rice Convention 2015 in May; and Thailand Lab 2015 in September.

Open Lab

Thailand Science Park, home of BIOTEC and its five research units, is considered the largest R&D community in Thailand, with government-funded research labs and also private labs of several companies. With such a concentration of facilities, Thailand Science Park makes an ideal study site for schools, universities, government and private organizations, as well as the general public. BIOTEC has an open-door policy and welcomes the public to tour laboratory facilities on a regular basis.

In 2015, 124 groups toured BIOTEC laboratories. Visitors included faculty members and students from various universities in Thailand; participants from the Thailand-UK Workshop on Plant-microbe Interactions; executives of Thailand Rice Department; and representatives of Japan External Trade Organization (JETRO) and Japanese Chamber of Commerce in Thailand.
The BIOTEC International Cooperation Program aims to capitalize on international links to help BIOTEC and Thailand become a regional leader in the field of biotechnology. In so doing, the Center has developed close relations with overseas organizations at the bilateral, multilateral and regional levels. These relations are developed through formal collaborative agreements, organizing joint scientific seminars with international partners, hosting foreign scientists and students in laboratories, and organizing an annual meeting of the BIOTEC International Advisory Board.
Fostering Collaboration

In fiscal year 2015, BIOTEC signed seven new Memorandums of Understanding (MOUs)/agreements and renewed one MOU to foster collaboration with the following organizations:

<table>
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<tr>
<th>Organization</th>
<th>Detail</th>
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<tbody>
<tr>
<td>Okinawa National College of Technology, Japan</td>
<td>To develop collaboration on cassava and starch technology.</td>
</tr>
<tr>
<td>Institut Pasteur, France</td>
<td>To study a vaccine candidate for porcine epidemic diarrhoea virus.</td>
</tr>
<tr>
<td>Earlham College, USA</td>
<td>To support the placement of students from Earlham College in BIOTEC laboratories</td>
</tr>
<tr>
<td>Institute of Microbiology, Chinese Academy of Sciences, China</td>
<td>To promote cooperation in the field of microbial biotechnology.</td>
</tr>
<tr>
<td>Food Industry Research and Development Institute, Taiwan</td>
<td>To foster cooperation in the field of microbial biotechnology.</td>
</tr>
<tr>
<td>Universiti Putra Malaysia, Malaysia</td>
<td>To promote cooperation in the field of microbial biotechnology.</td>
</tr>
<tr>
<td>Thermo Fisher Scientific Private Limited</td>
<td>To establish metabolomic and dereplication strategies for natural product research</td>
</tr>
<tr>
<td>Meijo University, Japan</td>
<td>To support research collaboration on salt-tolerant rice.</td>
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Technical Assistance for the Establishment of Culture Collection Center in Nepal

The advance in biotechnological and bioscience research enables the discovery of microbial utilization, especially in essential industries such as pharmaceuticals, energy and agriculture. Therefore, increasingly, culture collection becomes an important infrastructure for a bio-economy.

Nepal Academy of Science and Technology (NAST) is keen to establish a culture collection center as a part of their National Biotechnology Center. NAST recognized the expertise of BIOTEC in the management of microbial collections and thus contacted BIOTEC to seek collaboration on this topic. BIOTEC invited a scientific team from NAST, led by Dr. Buddhi Ratna Khadge, NAST Secretary, to visit Thailand on 24-26 August 2015 in order to learn first-hand of BIOTEC’s experience in establishing and managing its culture collection. The study visit focused on the management of microbial culture collection which includes activities such as the preservation, identification, quality control, data management, as well as legal management. NAST also had an opportunity to visit Thailand Institute of Scientific and Technological Research (TISTR) and KEEEN, a company specializing in bioremediation technology, to broaden their perspective on microbial collection and utilization.

BIOTEC will provide training to NAST research staff on culture collection management in 2016.

Thai-Lao Collaboration on Insect-pathogenic Fungi

In November 2014, an MOU on Thailand-Lao Cooperation on Science and Technology was entered into, with biotechnology listed among 14 fields of cooperation between the two nations. BIOTEC and its Lao counterpart, Biotechnology and Ecology Institute (BEI), have agreed to collaborate on research in biodiversity and ecology of insect-pathogenic fungi.

Under the collaborative framework, BIOTEC and BEI co-organized a Training Workshop on Collection, Identification and Preservation of Insect-pathogenic Fungi in Vientiane on 2-5 June 2015. The Workshop provided both fundamental knowledge and hands-on practice into the study of insect-pathogenic fungi. The Workshop was well attended by 15 research staff from BEI, National University of Laos and Plant Protection Center.
Thailand-China Silk Road Cooperation

BIOTEC and the Institute of Microbiology, Chinese Academy of Sciences (IMCAS) jointly organized a Seminar on Thailand-China Silk Road Cooperation on 19 May 2015, with 70 participants from research and academic organizations in Thailand. The seminar introduced the Chinese research and management of microorganisms and provided an opportunity for Thai and Chinese researchers to explore collaboration. The Chinese delegation was led by Prof. Shuang-Jiang Liu, Director-General of IMCAS. Topics of presentation included the CAS international cooperation, cooperation on microorganisms, China General Microbiology Culture Collection (CGMCC), WDCM global collaboration and yeast research.

On this occasion, BIOTEC entered into an MOU with IMCAS to promote research and capacity building in microbiology, especially on culture collection management, yeast, lactic bacteria, fungi and big data management of microorganisms.

Visit of Canadian Biopesticide Expert

BIOTEC invited Prof. Alan Watson, Director, Biopesticide Research Laboratory at McGill University, Canada to provide advice to BIOTEC’s biocontrol research and technology transfer program. His visit included a meeting with BIOTEC research team working on Beauveria bassiana and vegetative insecticidal proteins (Vips), inspection of project sites in Ayutthaya and Phrae provinces, to which the cultivation and spore production technology of B. bassiana developed by BIOTEC has been transferred, and a private company keen to develop a commercial Beauveria biopesticide.
International Exchange Programs

A number of programs have been established to host foreign researchers and students in BIOTEC laboratories. These are:

- **Human Resource Development Program in Biotechnology for Asia Pacific.** Operating since 2001, this program provides fellowships to young researchers from developing countries in the Asia-Pacific region to undergo research-based training in BIOTEC laboratories for a period of 3-6 months. Approximately 10-15 fellowships are granted annually.

- **TWAS-UNESCO Associateship Scheme.** Under this scheme, BIOTEC becomes one of 100 Centers of Excellence in the South to offer fellowships to foreign researchers from the South for training at, and fostering collaboration with, BIOTEC.

- **International Student Internship Program.** BIOTEC offers internship placements in laboratories for undergraduate and graduate students from overseas. This includes students sent from our partner institutes, under an established agreement, as well as students applying directly to the program. Current partner institutes include Atma Jaya Catholic University, Temasek Polytechnic, National Taiwan University, Nanyang Polytechnic, University of Kent, Soon Chun Hyang University and City University of Hong Kong. Earlham College joined this Program in 2015.

In 2015, BIOTEC hosted a total of 15 researchers and 58 students from 15 countries (Vietnam, Indonesia, Malaysia, Singapore, India, Taiwan, China, Korea, Japan, Germany, the Netherlands, the UK, the US, Canada, Mexico). Among the 73 exchange researchers/students, 8 visitors were on-going from the previous year.
IMPACT OF BIOTEC’S OUTPUT

Every year, a number of projects are selected for detailed impact study. These are projects that have created technologies or products that have actually reached the end users, through various forms of technology transfer such as technology licensing, provision of consultancy and training and establishing core infrastructure that would advance research in academic and industrial communities. Impact is measured in terms income generated by our clients from products and technologies and, where appropriate, assessed for the impact to the nation’s socio-economy in the forms of import substitution, investment and employment generation.
In 2015, 49 projects were selected for impact assessment. An estimated total of 5.27 billion Baht was generated from these projects. This amount was categorized as investment generation (127 million Baht), revenue generation to licensees or users (4.54 billion Baht), cost reductions (509 million Baht) and import replacement (91 million Baht).

**Agriculture and Food**

A total of 38 projects were evaluated, generating 1.54 billion Baht impact.

**New rice varieties.** This includes varieties that have been developed by the Rice Gene Discovery Laboratory, in collaboration with the Rice Department, namely blast-resistant glutinous rice (Thanayasirin) and flood-tolerant rice (Homcholasit). The two varieties were introduced to farmers in four regions of Thailand through the transfer of seed production technology. A total of 72 million Baht in income increment was estimated.

**Technologies for crop production.** BIOTEC engaged in various projects on plant improvement and technologies for crop production such as sugarcane breeding, increasing the production of disease-free sugarcane, orchid tissue culture production, rooftop and vertical plant cultivation system, environmentally-controlled greenhouse design, reagents for plant disease detection and the production of an NPV biopesticide product. These technologies have generated additional income to farmers and industries at the value of 232 million Baht.

**Animal and aquaculture production.** Technologies and products under the assessment include steroid-based synchronization of ovulation protocol for artificial insemination of cattle, shrimp breeding, a closed recirculating aquaculture system integrated with hybrid nitrification biofilter tanks and patented Tubular Denitrification Reactor (TDNR) and shrimp disease detection kits. These technologies have generated an impact of 350 million Baht.
Food and animal feed. Technologies that have been used at the commercial scale include starter culture for traditional fermented pork sausage, production technology of fermented soybean meal for animal feed, production of pentosanase for animal feed, industrial-scale production of pickle mustard greens using starter culture. These technologies have created income to the companies and investment to the economy at the value of 224 million Baht.

Community development. BIOTEC works with partners and local authorities to upgrade farming and cottage industry in the rural communities using area-based approach. Appropriate technologies, mainly related to farming and food processing, are made accessible to villagers with proper training. Examples of projects include promotion of organic rice production in Yasothorn province, agroforestry farming in Phrae province and Good Hygiene Practice (GHP) training for community food processing industries. These projects have improved the farming practice and food products, resulting in better quality produce and products. The impact from investment and additional income is estimated at 218 million Baht.

Remediation of saline land. The project was implemented in collaboration with SCG, Pimai Salt Co. Ltd., and various partners, using salt-tolerant varieties of tropical plants, as well as some economic crops screened and developed from BIOTEC laboratory, along with soil treatment with organics and minerals to treat saline land in four provinces - Sakon Nakhon, Udon Thani, Nakhon Ratchasima and Khon Kaen. The project was able to turn saline land into arable land for rice and other crops. Economic impact totaled 444 million Baht.
Health and Medicine

One project was evaluated and created an impact of 67 million Baht.

**Vaccine development.** Chimeric dengue virus strains developed by BIOTEC and partner universities were licensed to a company for further investigation and development into a commercial vaccine. This project has generated investment to the economy of 67 million Baht.

Environment

Seven projects were evaluated, resulting in an impact of 503 million Baht.

**Efficiency improvement in cassava starch processing.** Technology has been developed to improve the production efficiency in terms of energy and water consumption and raw material utilization in the cassava starch production process. The technology was introduced to cassava starch factories through practical training and advice to practitioners working in the factories. A total of 389 million Baht in savings was estimated from cost reduction and improved production yields.

**Biogas production from agro-industrial waste.** Wastewater treatment and biogas production technology was developed by EcoWaste, a joint lab between BIOTEC and King Mongkut’s University of Technology Thonburi. The technology has been implemented in agro-industry factories (cassava starch, palm oil mill, food processing), enabling wastewater compliance and energy cost savings of 78 million Baht.

**Environmentally-friendly products.** BIOTEC and a private company collaborated on a joint research project to develop a commercial bioremediation product based on oil-degrading microbes. The technology was subsequently licensed to the company for commercialization. Impact assessment was made on the revenue and the value of import substitution, totaling 36 million Baht.
Infrastructure Establishment

Three projects were evaluated resulting in an impact of 3.16 billion Baht.

Establishment of proteomics facility. This facility was established at Thailand Science Park, providing support on proteomics to researchers on a collaborative basis. The savings from seeking such a service from overseas were estimated to 82 million Baht.

Biopharmaceutical facility. This facility was established through collaboration between BIOTEC and King Mongkut’s University of Technology Thonburi (KMUTT) and is located at Bangkhuntien Campus of KMUTT. It offers contract manufacturing services for the manufacture of investigational drugs, vaccines and biopharmaceuticals for clinical research, as well as services on process optimization, scale up, fermentation processing development and downstream processing development. In this initial stage, the impact was assessed on the investment from private sector at 1 million Baht.

DNATEC Laboratory. The laboratory was jointly set up by BIOTEC and Kasetsart University, providing services in DNA fingerprinting to authenticate plant varieties. The authentication of jasmine rice and other commercial rice varieties facilitates the sale of rice and enables high quality varieties to command premium price. Impact of 3.07 billion Baht was estimated.
APPENDICES

List of Publications
List of Intellectual Properties
Honors and Awards
Executives and Management Team
List of Publications


### List of Intellectual Properties

#### List of Issued Intellectual Properties

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<tr>
<th>Title</th>
<th>Granted Date</th>
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<tr>
<td>Electrochemical detection of capsaicinoid compounds in a sample</td>
<td>3 February 2015</td>
<td>US 8,945,370 B2</td>
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<td>The production process and water supporting material made from cassava starch for tissue and cell culture</td>
<td>10 July 2015</td>
<td>44975</td>
<td>Thailand</td>
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<td>Patent</td>
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<tr>
<td>Method to screen and classify <em>Plasmodium falciparum</em> in blood sample</td>
<td>7 November 2014</td>
<td>9297</td>
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<td>Method to screen and classify <em>Plasmodium vivax</em> in blood sample</td>
<td>7 November 2014</td>
<td>9298</td>
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<td>Specific primers for shrimp parasite, <em>Enterocytozoon hepatopenaei</em>, and its application</td>
<td>29 December 2014</td>
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<td>Method to induce mutation in plant using the combination of controlled atmospheric pressure and mutagen</td>
<td>30 January 2015</td>
<td>9488</td>
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<td>Production process of recombinant lignocellulolytic enzymes by a slow methanol utilization strain of <em>Pichia pastoris</em></td>
<td>6 February 2015</td>
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<td>Simultaneous desizing and scouring process for natural fiber fabric using multi-activity enzymes</td>
<td>10 April 2015</td>
<td>9768</td>
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<td>Culture medium for growth of fungi isolated from marine and mangrove habitats</td>
<td>21 May 2015</td>
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<td>One-step desizing and scouring process of natural fiber fabric using multi-enzyme</td>
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<td>The use of yeast cell with phytase on cell surface in combination with a polysaccharide-degrading enzyme to improve nutrient in animal feed</td>
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<td>Pulp bleaching process using alkaline-tolerant xylanase obtained from metagenomic library of sugarcane bagasse compost</td>
<td>10 July 2015</td>
<td>10069</td>
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<td>Method for <em>Listeria monocytogenes</em> detection based on antibody array using liposome encapsulation conjugated with antibodies and enzyme for naked eye signal detection</td>
<td>10 July 2015</td>
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<td>Production process for enzyme - resistant rice starch type III</td>
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<td>Detection of infectious myonecrosis virus using immunochromatographic strip test</td>
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<td>Method to minimize released water and weight losses in fermented meat products under acidic condition using heat-modified whey protein isolates</td>
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<td>Inoculum preparation of methanol utilization slow (Mut^3) <em>Pichia pastoris</em> to improve fermentation efficiency</td>
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<td>Culture media to increase sporulation of blast pathogen</td>
<td>14 August 2015</td>
<td>10236</td>
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<td>Method to induce mutation in plant tissue culture under controlled environment</td>
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<td>Colorimetric detection method of methylparathion based on the interaction of gold nanoparticle and methylparathion hydrolase</td>
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<td>10306</td>
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<td>Method to induce mutation in plant using the combination of ultrasonic energy and mutagen</td>
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<td>Breeding flood-tolerant rice by mutation</td>
<td>28 August 2015</td>
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<td>Method to induce mutation in plant</td>
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<td>DNA vector for expressing gene responsible for the production of protein and metabolite in filamentous fungi</td>
<td>28 August 2015</td>
<td>10311</td>
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<td>Fermentation process of cassava feedstock with high solid content for the production of biofuels and chemicals</td>
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<td>Detection of white spot syndrome virus, WSSV, with LAMP-LFD technique</td>
<td>4 September 2015</td>
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<td>Non-autoclave tissue culture media</td>
<td>4 September 2015</td>
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<td>Enzymatic process for pulp grinding in the paper-making process</td>
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<td>Immunoassay based on flocculation of graphene oxide particles</td>
<td>11 September 2015</td>
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<td>Method to increase sporulation of rice-blast pathogenic fungus (<em>Magnaporthe grisea</em>) using organic solvents to extract metabolites from blast-infected leaves</td>
<td>18 September 2015</td>
<td>10412</td>
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<td>Immunochromatographic strip test for multiplex detection of three plant pathogens in cucurbits</td>
<td>18 September 2015</td>
<td>10414</td>
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<td>Plasmid vector for gene cloning in bacteria</td>
<td>18 September 2015</td>
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### List of Applied Intellectual Properties

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<tr>
<td>Cyclic peptide with broad spectrum inhibitions to human pathogens</td>
<td>22 September 2014</td>
<td>1401005578</td>
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<td>Virus-like particle for the induction of immune response against dengue virus infection and a method of generation</td>
<td>15 October 2014</td>
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<td>Specific primers and DNA probes for SNP biomarkers associated with the height of oil-palm trunk, and their applications</td>
<td>14 November 2014</td>
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<td>Dengue serotype 2 virus-based plasmids for the generation of codon-optimized, chimeric live attenuated vaccine candidates and the resultant viruses</td>
<td>28 November 2014</td>
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<td>Method to quantify the contamination of aflatoxin-producing mold</td>
<td>23 December 2014</td>
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<td>Bioreagent for one-step desizing and biostoning process of denim fabric</td>
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<td>Colloidal solution for acetone gas detection</td>
<td>13 February 2015</td>
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<td>Recombinant plasmid and recombinant Aspergillus sp. for the production of unsaturated fatty acid, dihomo-γ-linolenic acid</td>
<td>6 March 2015</td>
<td>1501001230</td>
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<td>Process and formulation of non-sodium and non-phosphate compounds for improving yield and textural quality of cooked chicken meat</td>
<td>12 March 2015</td>
<td>1501001462</td>
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<td>The process for the fabrication of polysalophen complex on an electrode for the electrochemical determination of artemisinin and products for a method thereof</td>
<td>26 May 2015</td>
<td>1501002871</td>
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<td>Improving extraction yield in cassava starch factory by recovery of starch in pulp at high solid loading with enzyme cocktails as a continuous integrated process</td>
<td>29 May 2015</td>
<td>1501002971</td>
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<td>Process of making cassava flour with high-viscosity for small scale production</td>
<td>29 May 2015</td>
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<td>Apparatus and method to enhance the emission of multicolor optical molecules</td>
<td>4 June 2015</td>
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<td>Anti-malarial and anti-cancer Phomoxdiene A</td>
<td>4 June 2015</td>
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<td>Steffimycin C, the potent antimycobacterial and antiplasmodial agent</td>
<td>12 June 2015</td>
<td>1501003280</td>
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<td>Screen printed electrode with surface modification for the determination of progesterone and the method thereof</td>
<td>10 July 2015</td>
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<td>Enzyme formulation for converting lignocellulosic biomass to sugar</td>
<td>29 July 2015</td>
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<td>Using image processing to automatically identify bands from gel electrophoresis image</td>
<td>3 August 2015</td>
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<td>Detection method for antifolate-resistant malaria pathogen</td>
<td>28 August 2015</td>
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<td>Culture media process for production of treholose by fermentation of thermotolerant yeast strain</td>
<td>28 August 2015</td>
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<td>Method of preparation of bacterial cellulose/recombinant staterin-fibronectin fusion protein / calcium phosphate composites</td>
<td>11 September 2015</td>
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<td>A method for genome-wide to identification of transcription start sites in eukaryotes from NGS transcriptome data</td>
<td>11 September 2015</td>
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<td>Crop improvement using colchicine and sodium azide by controlling light exposure-time</td>
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<td>Derivatives of 2,4-diamino-6-ethylpyrimidine inhibiting Plasmodium falciparum</td>
<td>23 September 2015</td>
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<td>Method of preparation of cellulose materials for cartilage tissue engineering using reductive amination reduction in acidic conditions</td>
<td>25 September 2015</td>
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<td>Protocol for isolation of live Acidovorax avenae subsp. citrulli from cucumber and watermelon using immunomagnetic separation in combination with selective medium</td>
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<td>Gene expression system for the production of target protein on yeast cell surface</td>
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<td>Preparation method of cassava starch-based hydrogel for use as disintegrant in drug tablet</td>
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<td>Recombinant yeast Pichia stipitis directly produces ethanol from cellulose, and its application</td>
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<td>Recombinant yeast Pichia stipitis directly produces ethanol from cellulose and hemicellulose, and its application</td>
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<td>Detection method for <em>Streptococcus agalactiae</em> in fish</td>
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<td>Detection method for <em>Aeromonas hydrophila</em> in fish</td>
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<td>Detection method for <em>Francisella noatensis</em> subsp. <em>orientalis</em> in fish</td>
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<td>Detection method for Infectious Spleen and Kidney Spleen and Kidney Necrosis Virus in fish</td>
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<td>Screening method for latent infection in sugarcane stalk</td>
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<td>Method to improve crop yield using the combination of high carbon dioxide treatment and mutagen</td>
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<td>Method to improve salinity and drought tolerance in soybean using the combination of plant growth regulator and mutagen</td>
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<td>Recombinant plasmid for extracellular expression of heterologous protein in yeast <em>Ogataea</em> spp.</td>
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<td>Method to improve crop yield in rice using the combination of high concentration sugar syrup, mutagen and ultrasonic energy</td>
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<td>Test kit for multi-drug resistant tuberculosis using nucleic acid lateral flow immunochromatography</td>
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<td>Method of preparing nanocomposite material from magnetic nanoparticles and cationic starch for chromium (VI) absorption</td>
<td>19 August 2015</td>
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<td>Culture media and cultivation process to enhance mycelium growth of <em>Boletus</em> mushroom</td>
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<td>Bioreagent for desizing and scouring process of fabric and its application</td>
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<td>The process of plant improvement for environmental stress conditions using direct current and mutagen</td>
<td>4 September 2015</td>
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<td>Method of preparation of porous cellulose materials for cell support</td>
<td>25 September 2015</td>
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<td>A marker recycling DNA tool for genome modification of yeast strain <em>Ogataea thermomethanolica</em> and its application</td>
<td>25 September 2015</td>
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<td>Immunomagnetic beads for specific isolation of <em>Acidovorax avenae</em> subsp. <em>citrulli</em></td>
<td>30 September 2015</td>
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<td>Monoclonal antibody to nucleocapsid protein of Tomato necrotic ringspot virus (TNRV) found in Thailand and its application in immunodetection of TNRV</td>
<td>30 September 2015</td>
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<td>Automatic thalassemia interpretation and couple at-risk assessment system for accurately identifying types of thalassemia from hemoglobin typing profiles</td>
<td>30 September 2015</td>
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<td>Solid cultured formulation for the preparation of A. niger BCC5639 inoculum</td>
<td>1 March 2015</td>
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<td>Method for multi-enzyme production from A. niger BCC5639</td>
<td>1 March 2015</td>
<td>N/A</td>
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</table>
Honors and Awards

Dr. Noppol Kobmoo
Bioresources Technology Research Unit
Marie Skłodowska-Curie Individual Fellowship to work with Prof. Titiana Giraud at the Ecology, Systematics and Evolution Laboratory of the University of Paris-Sud, France on a project entitled “Insights from Population Genomics to the Evolution of Host Specificity in Insect Fungi (GenoSpec)” for 2 years starting from November 2015 to 2017, awarded by Horizon 2020, the EU Framework Programme for Research and Innovation.

Dr. Atikorn Panya
Food Biotechnology Research Unit
In collaboration with King Mongkut’s University of Technology North Bangkok and the University of Massachusetts Amherst (USA)
The 2015 Edwin Frankel Award for Best Paper in Lipid Oxidation and Quality for the paper titled “Impact of free fatty acids and phospholipids on reverse micelles formation and lipid oxidation in bulk oil”, selected by the American Oil Chemists’ Society, (AOCS) to recognize the best paper relating to lipid oxidation or quality published during the past year in AOCS Press publications.

Dr. Chalermpol Kirdmanee
Genome Technology Research Unit
SCG Chair Professor Scholarship in recognition of his work on saline land rehabilitation, awarded by Siam Cement Group.

Dr. Bunpote Siridechadilok
Medical Biotechnology Research Laboratory
The 2014 Young Technologist Award in recognition of the development of virus construction that can save time and effort and enables the application of diverse genetic approaches, awarded by the Foundation for the Promotion of Science and Technology under the Patronage of His Majesty the King.

Dr. Theerayut Toojinda
Rice Gene Discovery Laboratory
The 2015 Plant Breeder Award in recognition of his success in utilizing marker-selected selection technique in rice improvement, presented by the Plant Breeding and Multiplication Association of Thailand.

Oil palm breeding and the development of tenera palm regeneration system through somatic embryogenesis
The 2015 Outstanding Project to recognize projects that have generated socio-economic impact, awarded by Agricultural Research Development Agency (Public Organization).

Ms. Wansika Kiatpathomchai, Dr. Rungkarn Suebsing and Ms. Jantana Kampeera
Biosensing Technology Research Unit
In collaboration with the National Center for Electronics and Computer Technology Center
The 2015 NRCT Invention Award (Agricultural Science and Biology) for “Portable rapid and low cost aflatoxin sensor with LAMP-Electrochemical detection kit”, awarded by the National Research Council of Thailand.

Dr. Nitsara Karoonuthaisiri, Dr. Rungnapa Leelatanawit, Ms. Umaporn Uawisetwathana, Ms. Thidathip Wongsurawat, Ms. Amornpan Klanchui and Ms. Natechanok Thamniedee
Biosensing Technology Research Unit and Animal Biotechnology Research Unit
The 2014 NRCT Research Award (Agricultural Science and Biology Field) for “The Development of DNA Microarray Technology to Address Poor Reproductive Maturation Problems in the Black Tiger Shrimp (Peneaus monodon)”, awarded by the National Research Council of Thailand.
**Dr. Phonphimon Wongthida**  
Animal Biotechnology Research Unit  
The 2014 NRCT PhD Dissertation Award (Medical Science Field) for “Understanding, and Exploiting, the Contribution of the Immune System to the Therapeutic Efficacy of Oncolytic Virotherapy with Vesicular Stomatitis Virus”, awarded by the National Research Council of Thailand.

**Dr. Peera Jaruampornpan**  
Animal Biotechnology Research Unit  
The 2014 NRCT PhD Dissertation Award (Chemistry and Pharmacy Field) for “Post - Translational Membrane Protein Targeting by the Chloroplast Signal Recognition Particle”, awarded by the National Research Council of Thailand.

**Dr. Pornkamol Unrean**  
Biochemical Engineering and Pilot Plant Research and Development Laboratory  
The 2014 TRF-CHE-Scopus Young Researcher Award (Engineering & Multidisciplinary Technology) for her work on “Systematic Bioprocess Development of Sustainable Biofuel Production”, awarded by the Thailand Research Fund (TRF), Office of the Higher Education Commission (CHE) and Elsevier.

**Ms. Phongphan Ek-arwut**  
Rural Development Technology Service Unit  
An award for her contribution to education in the monastic schools, presented by the National Office of Buddhism.
Executives and Management Team

Executive Board

Advisors
- Naksitte Coovattanachai: Advisor to Secretary-General, National Science Technology and Innovation Policy Office (STI)
- Morakot Tanticharoen: Professor Emeritus, School of Bioresources and Technology, King Mongkut’s University of Technology Thonburi
- Sermpol Ratasuk: Expert on organization planning and environmental impact assessment

Chairman
- Sakarindr Bhumiratana: President, King Mongkut’s University of Technology Thonburi

Vice Chairman
- Thaweesak Koanantakool: President, National Science and Technology Development Agency (NSTDA)

Members
- Porametee Vimolsiri: Deputy Secretary General, Office of the National Economic and Social Development Board (NESDB)
- Suwit Chaikiattiyos: Deputy Director-General, Department of Agriculture
- Chanun Puttaminprasethap: Deputy Director, Bureau of the Budget
- Prapon Wilairat: Professor Emeritus, Faculty of Science, Mahidol University
- Amaret Bhumiratana: Director, Royal Golden Jubilee Program, Thailand Research Fund (TRF)
- Julapark Chunwongse: Associate Professor, Faculty of Agriculture, Kasetsart University
- Pornsilp Patcharintanakul: Vice Chairman, Thai Chamber of Commerce
- Pachok Pongpanich: Executive Committee, Thai Seed Trade Association - THASTA
- Rutjawate Taharnklaew: Assistant Vice President, R&D Center, Betagro Group
- Kittiphong Limsuwannarot: Vice President of Green Chemicals, PTT Global Chemical Public Company Limited
- Kanyawim Kirtikara: Executive Director, BIOTEC
- Dussadee Siamhan: Deputy Executive Director, BIOTEC
International Advisory Board

**Chairman**
Lene Lange
Professor in Chemical and Biochemical Engineering, Technical University of Denmark, DENMARK

**Members**
Philippe Desmeth
President, World Federation for Culture Collections

Martin Keller
Associate Laboratory Director of Biological and Environmental Sciences, Oak Ridge National Laboratory (ORNL), USA

Vítor Martins dos Santos
Chair for Systems and Synthetic Biology, Wageningen University, THE NETHERLANDS

Ray Ming
Professor of Plant Biology, University of Illinois at Urbana-Champaign, USA

Jean-Marcel Ribaut
Director, Generation Challenge Programme (GCP)

Jonathan V. Sweedler
James R. Eiszner Family Chair in Chemistry, University of Illinois Urbana-Champaign, USA

Frédéric Tangy
Director of Research, French National Centre for Scientific Research (CNRS) and Head of the Viral Genomics and Vaccination Unit, Institut Pasteur, FRANCE

Management Team

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